THE EFFECT OF VARIATIONS IN THE LEVEL OF DIETARY CALCIUM UPON THE GROWTH OF YOUNG RATS RECEIVING ATABRINE

ANNE WILLIAMSON, D. M. HEGSTED, J. M. McKIBBIN, AND F. J. STARE

Department of Nutrition, Harvard School of Public Health, and the Department of Biochemistry, Harvard Medical School, Boston, Massachusetts

(Received for publication December 8, 1945)

Several interesting relationships between nutrition and the oral administration of atabrine have been demonstrated. Scudi and co-workers ('44) have shown that doses of atabrine administered by stomach tube were less toxic to animals fed a high-protein, low-fat diet than to animals receiving a low protein or a low-protein, high-fat diet. By incorporating atabrine in the ration, Hegsted, McKibbin and Stare ('44a) demonstrated retarded growth of young rats as well as some degree of liver damage when the level of atabrine in the ration exceeded 25 mg/100 gm ration (about 25 mg/kg body weight in weanling rats). Further studies with young rats showed that the inclusion of a relatively large proportion of atabrine in a ration deficient in choline prevents the development of hemorrhagic kidneys (Hegsted, McKibbin and Stare, '44b) and that atabrine exhibits a thiamine sparing action (Hegsted, McKibbin and Stare, '45).

Presented here are the results of studies in which the calcium content of the diet was modified and the effect upon the growth of young rats with and without additions of atabrine determined.

Copyright, 1946
The Wistar Institute of Anatomy and Biology
All rights reserved.

EXPERIMENTAL

Weanling male rats of the Sherman strain were housed in individual cages and fed the experimental rations ad libitum. Four to eight animals constituted a group. They were weighed twice weekly and during two studies records of food consumption over 4-week periods were made.

The basic purified ration was that described by Hegsted et al. ('44b). The calcium content of this ration (0.55%) was decreased to 0.15% in the low calcium rations by the substitution of 3% of a salt mixture low in calcium for the one previously used and was raised to 1.35% in the high calcium rations by the addition of 2% $CaCO_3$ at the expense of sucrose.

An alcohol solution of the water soluble vitamins was dried on the ration as described in the foregoing publications and fat soluble vitamins in corn oil were given by dropper twice weekly.[1] Atabrine hydrochloride dissolved in alcohol was added to the ration at levels of 40 or 60 mg per 100 gm.

Determination of atabrine in liver and spleen was made by extracting a tissue homogenate (McIlvaine's buffer, pH 7.8) with ethylene chloride; atabrine was extracted from this with 0.3 N HCl for reading in the photofluorometer.

The pH of the intestinal content of certain of the experimental animals was determined as follows: the animals were sacrificed individually and the intestinal tract excised and divided into three segments, upper and lower small intestine and cecum. The contents of each of these portions were then washed immediately into a small beaker with 20 ml distilled water and the pH determined by means of a glass electrode. Values found for the lower small intestine and cecum were consistent for animals on a given diet; in the upper portion of the tract the range of variation was somewhat greater and stomach pH figures were discarded because of the extreme variation found, presumably due to differences in time since the ingestion of food.

[1] We are indebted to Merck & Co., Inc., Rahway, New Jersey, for furnishing the crystalline B-vitamins and alpha tocopherol, and to Abbott Laboratories, North Chicago, Illinois, for halibut liver oil.

RESULTS AND DISCUSSION

As in the experiments cited previously, the addition of 40 to 60 mg of atabrine per 100 gm purified ration resulted in a decrease of 20 to 30% in the weight gain of young rats (table 1). It may be seen that alterations in the level of dietary calcium intake were accompanied by differences in tolerance to atabrine as measured by growth. In the series of four experiments, rats receiving 40 or 60 mg % atabrine with the high calcium diet attained but 51%–63% of the weight gain of their respective controls not receiving atabrine. On the other hand, animals receiving the same amount of atabrine in the ration but maintained at the lower calcium levels grew 67%–77% as well as the appropriate control groups. Statistical treatment of these data by means of the "t test" showed these differences significant with the possible exception of experiment 2.

Food intake records (table 2) show that the rats receiving atabrine consumed somewhat less than the control groups; however the efficiency of food utilization was variable. The actual amount of atabrine ingested by the low and high calcium groups of animals was comparable, about 4 mg per day. Despite the fact that the low and high calcium groups ingested the same amount of atabrine, determinations of atabrine concentration in liver and spleen showed a difference between the two groups. It may be seen from table 3 that rats on the high calcium diet stored on the average twice the amount of atabrine in the liver as did animals at the low calcium level. These differences again were found to be statistically significant. The concentration of atabrine in the spleen reflected the same trend although the contrast between the two groups was less marked.

It is observed that the atabrine concentration in the livers of groups receiving either the high or low calcium ration was no higher after 63 days on the diet than after only half that time. The concentration of atabrine in the liver varied with the individual animal and with the calcium level of the diet. In these experiments it appeared to be attained within a com-

TABLE 1

Average gain in weight of young rats on various levels of calcium, atabrine, and other supplements.

EXPERIMENT NO.	RATION			NO. ANIMALS	DURATION OF EXPERIMENT	AVERAGE WEIGHT GAIN	PLUS ATABRINE AS % OF CONTROLS
	Calcium	Atabrine	Supplement				
	%	*mg/100 gm ration*			*days*	*gm*	
1	0.15	..	None	5	63	223	..
	0.15	40	None	4		173	77
	0.55	..	None	4		216	..
	0.55	40	None	5		147	68
	1.35	..	None	4		247	..
	1.35	40	None	5		125	51
2	0.15	..	None	6	51	155	..
	0.15	60	None	5		105	67
	0.15	..	6% ammonium salt mixture [1]	5		144	..
	0.15	60	6% ammonium salt mixture	5		85	59
	1.35	..	None	6		164	..
	1.35	60	None	5		95	58
	1.35	..	6% ammonium salt mixture	5		140	..
	1.35	60	6% ammonium salt mixture	5		82	59
3	0.15	..	None	6	46	171	..
	0.15	40	None	6		121	71
	0.15	..	2% citric acid	6		149	..
	0.15	40	2% citric acid	5		104	70
	1.35	..	None	4		162	..
	1.35	40	None	6		102	63
	1.35	..	2% citric acid	5		157	..
	1.35	40	2% citric acid	6		115	73
4	0.15	..	None	7	30	110	..
	0.15	40	None	8		80	73
	1.35	..	None	7		97	..
	1.35	40	None	8		57	58
	0.15	..	1% $MgCO_3$	3		82	..
	0.15	40	1% $MgCO_3$	3		58	71

[1] Two per cent $NaHCO_3$ was used instead of the ammonium salts for 30 days; when it was found that there was no effect either upon growth or the intestinal reaction, the mixture of equal parts ammonium carbonate and ammonium chloride was substituted at a 6% level.

paratively short period of time and was not influenced by long-continued ingestion of atabrine. It might be suggested, therefore, that the differences in tissue concentration and growth rate manifested by animals at different calcium levels were due less to changes in absorption of atabrine from the intestinal tract than to some factor influencing the fate of the drug after entering the body.

TABLE 2

Food consumption and efficiency of food utilization on varying levels of calcium and atabrine.

RATION	AVERAGE FOOD INTAKE		EFFICIENCY OF FOOD UTILIZATION	
	Experiment 1	Experiment 4	Experiment 1	Experiment 4
	gm/day	*gm/day*	*gm food/gm gain*	*gm food/gm gain*
0.15% calcium	12.1	10.4	2.8	2.8
0.15% calcium + 40 mg % atabrine	10.9	9.5	3.5	3.4
0.55% calcium	13.4	....	3.4	...
0.55% calcium + 40 mg % atabrine	10.5	....	3.3	...
1.35% calcium	15.5	14.0	3.4	4.2
1.35% calcium + 40 mg % atabrine	11.2	8.6	4.0	4.2
0.15% calcium + 1% $MgCO_3$	....	6.9	...	2.7
0.15% calcium + 1% $MgCO_3$ + 40 mg % atabrine	....	5.3	...	2.8

In this connection it should be stated that several of the animals on the high calcium ration without atabrine were found to have suffered renal damage. The kidneys were hypertrophied and numerous stones were observed in the kidneys and in the bladder. It is possible that the rats on the high calcium ration were unable to excrete as much atabrine via this route as was the group on the low calcium diet.

In experiments 2, 3, and 4 an attempt was made to determine whether or not the effect of the calcium content of the diet in establishing the degree of atabrine toxicity might be due to altered pH of the intestinal contents. It is known that a high proportion of calcium in a purified diet promotes a

more alkaline intestinal reaction. Substances thought to produce acidic or basic reaction were added to the low and high calcium rations and pH determinations made on the intestinal contents of animals fed these diets for a week or more. Results in terms of the pH of the intestinal contents are presented in table 4. Of the substances tested, only

TABLE 3

Concentration of atabrine in liver and spleen of rats receiving 40 mg % atabrine on low and high calcium diets.

EXPERIMENT NUMBER	NO. ANIMALS	DURATION OF EXPERIMENT	ATABRINE			
			Liver		Spleen	
			0.15% Ca	1.35% Ca	0.15% Ca	1.35% Ca
		days	*γ/gm*	*γ/gm*	*γ/gm*	*γ/gm*
1	4	63	235	2000	870	1520
			872	1525	1170	1310
			520	1300	1040	1180
			450	1000	730	1180
Average			519	1456	953	1298
4	8	30	631	1360		
			733	1904		
			593	1070		
			721	1366		
			528	1624		
			1275	1289		
			1147	1520		
			709	1675		
Average			792	1476		

$MgCO_3$ and the $CaCO_3$ of the high calcium ration produced an alkalinity of the intestine, and that was apparent only in the lower small intestine and cecum. The inclusion of atabrine in a ration did not affect the intestinal pH of the animal.

The growth data for rats receiving citric acid, a mixture of equal parts of ammonium chloride and ammonium carbonate, or magnesium carbonate in addition to the high and low calcium levels with and without atabrine are included in

table 1. It may be seen that the ammonium salts did not affect the response to the high calcium ration but did increase somewhat the growth inhibition due to atabrine on the low calcium ration. The citric acid, conversely, improved the high calcium ration so that rats receiving atabrine plus high calcium with citric acid grew as well as did the low calcium animals receiving atabrine, with or without citric acid. This may indicate that acidity or alkalinity of the diet influences

TABLE 4

The pH of intestinal contents of rats maintained on varying levels of calcium and with certain salt supplements.

CALCIUM CONTENT OF RATION	SUPPLEMENTS	NO. OF ANIMALS	pH OF INTESTINAL CONTENTS		
			Upper small intestine	Lower small intestine	Cecum
0.15%	None	16	6.34 ± 1.02	6.64 ± 0.32	6.73 ± 0.28
	1% $NaHCO_3$	2	6.28	6.63	6.62
	1% $Al(OH)_3$	2	5.95	6.87	6.60
	1% $MgCO_3$	2	6.24	7.63	8.05
	3% NH_4Cl + 3% $(NH_4)_2CO_3$	6	6.19	6.79	6.61
	2% citric acid	5	6.45	6.74	6.89
0.55%	None	2	6.54	6.95	7.40
	1% citric acid	2	6.78	7.19	7.32
	2% citric acid	2	6.63	7.04	7.28
1.35%	None	15	6.53	7.58	7.95
	3% NH_4Cl + 3% $(NH_4)_2CO_3$	2	6.60	8.01	8.19
	2% citric acid	5	6.51	7.89	8.26

atabrine absorption or storage, although citric acid and the ammonium salt mixture could not be shown to influence the pH in the intestinal tract. A small group of animals given the low calcium ration to which 1% $MgCO_3$ was added (experiment 4) showed no significant growth difference in response to atabrine from the low calcium controls. However, $MgCO_3$ was shown to produce an intestinal reaction as alkaline as that found on the high calcium diet. It is not clear, therefore,

whether the calcium ion exerts some specific effect upon the fate of ingested atabrine, whether the discrepancies were due to defective excretion, or whether the augmented toxic effect observed on the high calcium diet is in some way associated with the intestinal pH.

A few attempts were made to study in vitro the intestinal absorption of atabrine by passing solutions of the drug through an isolated loop of fresh intestinal tissue suspended in a buffer medium. Comparison with xylose diffusion curves conducted simultaneously suggest that, under the conditions imposed, atabrine passed through the membrane by diffusion. It was of interest that characteristic peristaltic movements continued in the isolated intestinal loop for more than an hour after the addition of atabrine. Tissue similarly placed in the buffer but without atabrine did not exhibit this action. This observation is of interest as Keogh and Shaw ('44) have reported that the action of calcium ions in causing the relaxation of smooth muscle is reversed in the presence of atabrine or quinine. Gastrointestinal symptoms following administration of atabrine to man have been reported (Goodman and Gilman, '41).

CONCLUSIONS

1. The inhibition of growth of young rats by the addition of 40 or 60 mg % atabrine to a purified ration was significantly greater in animals maintained on a high calcium ration, 1.35%, than when the ration contained a calcium level of 0.55% or 0.15%.

2. The average concentration of atabrine in the liver and spleen of rats on the high calcium diet was considerably greater than that found for animals receiving the low calcium diet, despite a comparable atabrine intake.

3. The concentration of atabrine in the liver in these experiments was as high at 30 days as after 63 days.

4. Animals on the high calcium diet had a more alkaline reaction in the lower small intestine and cecum. However, by the addition of various salts it could not be shown that in-

testinal pH was a major factor in affecting atabrine toxicity. Some animals on the high calcium diet exhibited renal calculi which may have impaired the excretion of atabrine. Hence it is not clear whether the increased toxicity of atabrine on a high calcium intake is due to increased absorption, decreased excretion, or to a specific effect of calcium on atabrine metabolism.

LITERATURE CITED

GOODMAN, L., AND A. GILMAN 1941 The Pharmacological Basis of Therapeutics. New York, Macmillan Company.

HEGSTED, D. M., J. M. MCKIBBIN AND F. J. STARE 1944a Nutrition and tolerance to atabrine. J. Nutrition, vol. 27, p. 141.

———— 1944b The effect of atabrine on choline deficiency in the young rat. J. Nutrition, vol. 27, p. 149.

———— 1945 The effect of atabrine on thiamine deficiency in the young rat. J. Nutrition, vol. 29, p. 361.

KEOGH, P. P., AND F. H. SHAW 1944 The mode of action of quinine alkaloids and other anti-malarials. Austr. J. Exp. Biol. Med. Sci., vol. 22, p. 139.

SCUDI, J. V., V. C. JELINEK AND S. KUNA 1944 Biochemical aspects of the toxicity of atabrine. II. Influence of the diet on the effects produced by repeated doses of atabrine. J. Pharmacol., vol. 80, p. 144.

PHOSPHORUS METABOLISM OF PRESCHOOL CHILDREN

BEULA V. McKEY, MARIE FOLSOM CLARK, FRIEDA L. MEYER AND MILICENT L. HATHAWAY

New York State College of Home Economics, Cornell University, Ithaca

(Received for publication January 11, 1946)

This report on phosphorus metabolism is the fourth in a series on the effect of changes in the ascorbic acid and citrate content of the diet on the ascorbic acid, citric acid, calcium, phosphorus, and nitrogen metabolism of eight preschool children. The effect on ascorbic acid, citric acid, and calcium metabolism have been reported by Meyer and Hathaway ('44), Metcalf and Hathaway ('45) and Watson and associates ('45), respectively.

EXPERIMENTAL

The general plan of the complete experiment has been reported in detail by Meyer ('43), and a summary of the facts pertinent to the calcium and phosphorus metabolism studies given by Watson and associates ('45). Briefly, they are these: eight children between the ages of 38 and 55 months and weighing 29 to 50 pounds were maintained on a basal diet believed to be adequate in all nutrients except ascorbic acid. The first year the children A, B, C, and D were given 800 ml of milk daily; the second year, subjects E, F, G, and H received only 500 ml. Through changes in the basal diet, particularly an increase in the meat and eggs, an attempt was made to maintain the level of phosphorus in the low-milk diet. There was greater variation from subject to subject in the phosphorus intakes on the low-milk diet, however, be-

cause these changes were in the basal diet, and somewhat less of the basal foods was consumed by subject H throughout the study, and by subject G during the first 4 and last 5 weeks. Also, the Ry-Krisp and Toasted Wheat Wafers, allowed ad libitum, contained significant amounts of phosphorus. Supplements of crystalline ascorbic acid (100 mg), potassium citrate (3.38 gm), or both were given as indicated in table 1. During the last 2 weeks, the children of the low-milk group were given orange juice in place of crystalline supplements.

The methods of collecting and preparing samples for analysis have been described by Metcalf and Hathaway ('45) and Watson and associates ('45). With the exception of the milk samples for the second year, all samples for phosphorus analyses were ashed using essentially the method of Gerritz ('35). The phosphorus content of the ashed samples was determined by a modification of the method of Fiske and Subbarow ('25), adapting it to the photoelectric colorimeter. Exact timing for the development of the color in samples and standards was used.

The milk samples for the second year were ashed in a muffle furnace at about 400°C., as suggested by Peters and Van Slyke ('32). The phosphorus values were much lower by this method, and are believed to be unreliable. The milks for the 2 years were specially prepared in the University Dairy Department.[1] They were obtained from the same herd, at the same time of year, and had the same average nitrogen and calcium content. Since the calcium : phosphorus ratio in milk under these conditions should be constant, it seemed advisable to use the average found for phosphorus in milk for the first year in calculating the phosphorus intakes for the second year.

RESULTS AND DISCUSSION

The data obtained in this study are presented in table 1. Data for the four preliminary periods have been omitted.

[1] Specially prepared by Prof. E. S. Guthrie to preserve the ascorbic acid content. See Sharp, Hand, and Guthrie ('39).

TABLE 3

Average daily phosphorus balances for eight preschool children.

DIET	PERIOD	SUBJECT A				SUBJECT B				SUBJECT C				SUBJECT D			
		INTAKE	EXCRETION Urine	EXCRETION Feces	RETENTION	INTAKE	EXCRETION Urine	EXCRETION Feces	RETENTION	INTAKE	EXCRETION Urine	EXCRETION Feces	RETENTION	INTAKE	EXCRETION Urine	EXCRETION Feces	RETENTION
		mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg
I [1]	5	....	...	.		1249	750	434	65	1258	670	454	134	1234	777	427	30
	6	1374	537	631	206	1343	690	404	249	1366	662	447	257	1350	739	415	196
	10	1321	607	605	109	1306	742	410	154	1321	774	418	129	1271	724	433	114
	11	1294	588	578	128	1245	702	430	113	1296	727	422	147	1235	753	346	136
	12	1208	515	539	154	1168	693	403	72	1203	631	406	166	1168	747	368	53
	Av.	1299	562	588	149	1262	715	416	131	1289	693	429	167	1252	748	398	106
II [2]	7	1299	578	578	143	1266	711	417	138	1299	626	412	261	1257	782	357	118
	8	1303	569	601	133	1285	698	394	193	....	...	.		1261	748	363	150
	9	1309	519	638	152	1292	718	414	160	1312	753	406	153	1270	734	359	177
	13	1254	605	545	104	1212	725	390	97	1237	729	395	113	1217	759	339	119
	14	1297	614	547	136	1235	725	373	137	1266	715	491	60	....	...	...	...
	Av.	1292	577	582	134	1258	715	398	145	1282	732	438	112	1251	756	354	141
III [3]	15	1270	609	369	292	1237	779	546	—88	1257	647	431	179	1246	700	350	196
	16	1307	637	546	124	1267	719	404	144	1289	748	431	110	1268	787	325	156
	17	1306	591	538	177	1268	705	408	155	1299	631	565	103	1275	664	400	211
	Av.	1294	612	484	198	1257	734	453	70	1282	675	476	131	1263	717	358	188
IV [4]	18	1264	617	543	104	1226	708	379	139	1253	738	447	68	1228	720	332	176
	19	1281	622	517	142	1248	736	399	113	1262	692	477	93	1249	739	360	150
	20	1262	592	547	123	1227	663	481	83	1255	697	485	73	1240	752	357	131
	Av.	1269	610	536	123	1234	702	420	112	1257	709	470	78	1239	737	350	152
		SUBJECT E				SUBJECT F				SUBJECT G				SUBJECT H			
I [1]	7	1077	580	415	82	1077	601	388	88	1077	578	409	90	961	521	335	105
	8	1089	576	433	80	1085	588	388	109	1085	540	448	97	982	535	334	113
	9	1114	588	446	80	1109	586	440	83	1109	566	427	116	998	548	327	123
	Av.	1093	581	431	81	1090	592	405	93	1090	561	428	101	980	535	332	114
II [2]	5	1120	599	455	66	1131	597	409	125	1135	534	494	107	985	513	368	104
	6	1080	573	474	33	1076	592	415	69	1087	536	490	61	956	524	363	69
	10	1082	601	430	51	1078	567	419	92	1071	564	415	92	977	547	317	113
	11	1091	602	398	91	1087	576	436	75	1037	540	398	99	967	529	493	—55
	12	1099	594	415	90	1095	593	458	44	1066	564	419	83	990	548	325	117
	Av.	1094	594	434	66	1093	585	427	81	1079	548	443	88	975	532	373	70
III [3]	13	1096	610	415	71	1092	596	404	92	1039	539	366	134	988	534	229	225
	14	....	...	.		1084	570	352	162	1019	547	363	109	976	513	352	111
	15	1101	600	415	86	1093	588	424	81	1010	560	343	107	971	514	337	120
	Av.	1098	605	415	78	1090	585	393	112	1023	549	357	117	978	520	306	152
IV [4]	16	1096	593	419	84	1091	583	442	66	901	522	339	40	951	534	335	82
	17	1094	595	430	69	1097	567	433	97	896	529	320	47	948	518	352	78
	18	1106	580	404	122	1100	565	419	116	905	500	322	83	952	505	322	125
	Av.	1099	589	418	92	1096	572	431	93	901	517	327	57	950	519	336	95
V [5]	19	1129	613	384	132	1118	605	421	92	928	534	301	93	950	527	310	113
	20	1139	635	382	122	1127	607	393	127	922	520	314	88	940	554	301	85
	Av.	1134	624	383	127	1122	606	407	109	925	527	307	91	945	540	305	99

[1] Basal diet only. [2] Basal diet plus ascorbic acid. [3] Basal diet plus potassium citrate. [4] Basal diet plus ascorbic acid and potassium citrate. [5] Basal diet plus orange juice.

Summaries of the phosphorus balances in mg/kg of body weight and of the percentages of phosphorus retained are given in table 2.

Phosphorus intake and retention. Phosphorus metabolism has received less attention than that of calcium and nitrogen, probably because the phosphorus needs are satisfied if the calcium and protein needs are adequately met through common foods. The National Research Council's recommended dietary allowances ('45) do not include values for phosphorus, but suggest that the phosphorus of children's diets should equal or exceed the calcium content. In this study these conditions have been met since the calcium: phosphorus ratios for the four children on the high-milk intake ranged from 0.86 to 0.91, and for the low-milk group from 0.69 to 0.82. The phosphorus intakes for the eight children ranged from 896 to 1374 mg, or from 55 to 76 mg/kg of body weight. These intakes are similar to those given in other reports on phosphorus metabolism of preschool children (Porter-Levin, '33, '33–'34; Daniels et al., '35, '37; Pierce et al., '40; and Hawks et al., '42).

Most of the reports of phosphorus retentions by preschool children are made in terms of mg/kg/day. Porter-Levin ('33–'34) reported average retentions of 5, 5, and 8 mg/kg; Daniels et al. ('35) 6–9 mg; Pierce et al. ('40), 6.9 to 7.4 mg/kg; and Hawks et al. ('42), 3 to 8 mg/kg. The average retentions for the eight subjects of this study ranged from 3.5 to 10.8 mg/kg on the various diets used.

Macy ('42) states that 48 to 65% of the ingested phosphorus is excreted in the urine, suggesting the wide use of phosphorus in metabolism. It also suggests that the supply of phosphorus to the tissues is generally ample, or smaller excretions would be found. The phosphorus excretions for the eight children in this study are in line with those reported in the literature, and indicate adequate supply even on the lower intakes.

Effect of ascorbic acid supplement on phosphorus retention. Daniels and Everson ('37) found no significant changes in phosphorus retention with variations in ascorbic acid intake,

TABLE 2

Summary of phosphorus balances of eight preschool children.

DIET	INTAKE	EXCRETION		RETENTION	
		Urine	Feces		
	mg/kg	*mg/kg*	*mg/kg*	*mg/kg*	*% of intake*
			Subject A		
I [1]	57	25	26	6.6	11
II [2]	57	26	26	5.9	10
III [3]	57	27	21	8.6	15
IV [4]	55	27	23	5.3	10
			Subject B		
I [1]	76	43	25	7.9	10
II [2]	74	43	24	8.7	12
III [3]	73	43	26	4.1	6
IV [4]	71	41	24	6.5	9
			Subject C		
I [1]	72	39	24	9.4	13
II [2]	70	40	24	6.2	9
III [3]	68	36	25	6.9	10
IV [4]	66	38	25	4.1	6
			Subject D		
I [1]	75	45	24	6.3	8
II [2]	74	44	21	8.3	11
III [3]	71	40	20	10.6	15
IV [4]	69	41	19	8.4	12
			Subject E		
I [1]	68	36	27	5.0	7
II [2]	68	37	27	4.1	6
III [3]	66	36	25	4.6	7
IV [4]	65	35	25	5.4	8
V [5]	66	36	22	7.3	11
			Subject F		
I [1]	59	32	22	5.0	9
II [2]	58	31	23	4.3	7
III [3]	57	31	21	5.9	10
IV [4]	56	29	22	4.7	8
V [5]	57	31	21	5.5	10
			Subject G		
I [1]	69	36	27	6.4	9
II [2]	68	34	28	5.5	8
III [3]	62	33	22	7.0	11
IV [4]	55	31	20	3.5	6
V [5]	56	32	19	5.5	10
			Subject H		
I [1]	71	39	24	8.3	12
II [2]	71	39	27	5.1	7
III [3]	69	37	22	10.8	16
IV [4]	66	36	23	6.6	10
V [5]	66	37	21	6.9	10

[1] Basal diet only.
[2] Basal diet plus ascorbic acid.
[3] Basal diet plus potassium citrate.
[4] Basal diet plus ascorbic acid and potassium citrate.
[5] Basal diet plus orange juice.

but Shepherd et al. ('40) report decreases of 1.4 mg/kg/day on the addition of 20 mg of ascorbic acid. The decrease was ascribed to increases in the urinary excretion of phosphorus. In the eight children of the present study when 100 mg ascorbic acid were added, the phosphorus retentions were decreased for five, increased for one, and showed little difference for two of the subjects, but the differences in retention with and without the supplement were not significant for any child.

Effect of potassium citrate supplement on phosphorus retention. Comparison of the average retentions of phosphorus when 3.38 gm of potassium citrate were added to the diet, with the retentions without this supplement, shows that the phosphorus retentions were increased for four children, decreased for three, and unchanged for one with the supplement. The differences in retention were not significant for any child.

Effect of orange juice supplement on phosphorus retention. Approximately 218 ml of orange juice were added to the basal diet of subjects E, F, G, and H during the last 2 weeks of the study. The data for these periods are included in tables 1 and 2 under diet V. For subject E the retentions of phosphorus on this supplement were the greatest found at any time, but for the other three, the differences in retention were not appreciable. Chaney and Blunt ('25) have reported increased phosphorus retention in two adolescent girls; Daniels and Everson ('37) reported no significant differences in retention for three preschool children; and Shepherd et al. ('40) reported decreased retention in 5 young children when fresh orange juice was added to the diet. The results of the present study support the evidence of Daniels and Everson that there is no significant effect of addition of orange juice on the retention of phosphorus in preschool children.

SUMMARY AND CONCLUSIONS

Phosphorus metabolism has been studied in eight preschool children, on phosphorus intakes of 896 to 1374 mg/day, or

55 to 76 mg/kg/day. The values for retentions per kilogram of body weight were in line with those reported in the literature, average values varying from 3.5 to 10.8 mg/kg. The basal diet was supplemented with ascorbic acid, potassium citrate, and orange juice as indicated. None of the supplements caused significant alterations in phosphorus retentions, although rather wide variations occurred from period to period. This might indicate that other factors than diet are largely responsible for fluctuations in phosphorus retentions.

LITERATURE CITED

CHANEY, M. S., AND K. BLUNT 1925 The effect of orange juice on the calcium, phosphorus, magnesium, and nitrogen retention and urinary organic acids of growing children. J. Biol. Chem., vol. 66, p. 829.

DANIELS, A. L., AND G. J. EVERSON 1937 The relation of ascorbic acid ingestion to mineral metabolism in children. J. Nutrition, vol. 14, p. 317.

DANIELS, A. L., M. K. HUTTON, E. M. KNOTT, O. E. WRIGHT AND M. FORMAN 1935 Calcium and phosphorus needs of preschool children. J. Nutrition, vol. 10, p. 373.

FISKE, C. H., AND Y. SUBBAROW 1925 The colorimetric determination of phosphorus. J. Biol. Chem., vol. 66, p. 375.

GERRITZ, H. W. 1935 Digesting biological materials for calcium and phosphorus analysis. Ind. Eng. Chem., Anal. Ed., vol. 7, p. 167.

HAWKS, J. E., M. M. BRAY, M. O. WILDE AND M. DYE 1942 The interrelationship of calcium, phosphorus and nitrogen in the metabolism of preschool children. J. Nutrition, vol. 24, p. 283.

MACY, I. G. 1942 Nutrition and Chemical Growth in Childhood. Vol. 1. Evaluation. Charles C. Thomas, Springfield, Ill.

METCALF, E. R., AND M. L. HATHAWAY 1945 Citrate metabolism of preschool children. J. Nutrition, vol. 29, p. 211.

MEYER, F. L. 1943 Ascorbic acid requirement and metabolism of preschool children. Thesis, Cornell University.

MEYER, F. L., AND M. L. HATHAWAY 1944 Further studies on the vitamin C metabolism of preschool children. J. Nutrition, vol. 28, p. 93.

NATIONAL RESEARCH COUNCIL 1945 Recommended dietary allowances. Bull. National Research Council, no. 122 (Aug.).

PIERCE, H. B., R. G. DAGGS, A. B. MESERVEY AND W. J. SIMCOX 1940 The retention of calcium and phosphorus by preschool children. J. Nutrition, vol. 19, p. 401.

PETERS, J. P., AND D. D. VAN SLYKE 1943 Quantitative Clinical Chemistry. Vol. 2. Methods. Williams and Wilkins.

PORTER-LEVIN, T. 1933 Calcium and phosphorus metabolism of normal preschool children: I. On diets containing plain and irradiated cereals. J. Am. Dietetic Assoc., vol. 8, p. 482.

PORTER-LEVIN, T. 1933–1934 II. Successive balance studies showing the range of variation in calcium and phosphorus storage. J. Am. Dietetic Assoc., vol. 9, p. 22.

SHARP, P. F., D. B. HAND AND E. S. GUTHRIE 1939 Preliminary report on the deaeration of market milk. Thirteenth Annual Report of N. Y. State Assoc. of Dairy and Milk Inspectors, p. 95.

SHEPHERD, M. L., I. G. MACY, H. A. HUNSCHER AND F. C. HUMMEL 1940 Synthesized, processed and natural sources of vitamin C in the mineral metabolism of normal children. J. Ped., vol. 16, p. 704.

WATSON, E. K., E. W. MCGUIRE, F. L. MEYER AND M. L. HATHAWAY 1945 Calcium metabolism of preschool children. J. Nutrition, vol. 30, p. 259.

NITROGEN METABOLISM OF PRESCHOOL CHILDREN

MARION S. LEW, DOROTHY D. CLAGETT, FRIEDA L. MEYER AND MILICENT L. HATHAWAY

New York State College of Home Economics, Cornell University, Ithaca

(Received for publication January 11, 1946)

Studies by Daniels and Everson ('37) and Shepherd et al. ('40) present conflicting data on the effect of changes in the ascorbic acid content of the diet on the nitrogen retention by preschool children. This report presents data on the nitrogen metabolism of eight preschool children whose diet was supplemented with ascorbic acid, potassium citrate, and orange juice. The effect of these supplements on ascorbic acid, citric acid, calcium, and phosphorus metabolism has been reported in previous papers by Meyer and Hathaway ('44), Metcalf and Hathaway ('45), Watson and associates ('45), and McKey and associates ('46).

EXPERIMENTAL

Two groups of four children each lived at the college laboratory apartment for 5 months. Subjects A, C, F, G, and H were girls, aged 55, 49, 49, 40, and 38 months, respectively, at the beginning of the study. Their respective weights at that time were 50, 38, 40, 32, and 29 pounds. Subjects B, D, and E were boys, aged 51, 44, and 55 months, and weighing 36, 35, and 34 pounds, respectively.

The general plan of the complete experiment, including a description of all subjects and of the diets used, has been reported in detail by Meyer ('43), and summarized in the previous papers of this series. The following facts are partic-

ularly pertinent to this study. The children were maintained on a basal diet adequate in all nutrients except ascorbic acid. The first group of children (A, B, C, and D) were given 800 ml of milk daily; the other group (E, F, G, and H) received only 500 ml. Through an increase in the meat, eggs, and peanut butter similar levels of protein were maintained in the basal diets of the two groups. Ry-Krisp or Toasted Wheat Wafers were allowed ad libitum so that there was some variation in nitrogen intake from period to period for a given child and from child to child for subjects A through F. Somewhat less of the basal foods was consumed by subject H throughout the study, and by subject G during the preliminary weeks and during periods 16 through 20, resulting in lower nitrogen intakes for these two girls. Supplements of crystalline ascorbic acid (100 mg), potassium citrate (3.38 gm) or both were given as indicated in table 1. These amounts correspond roughly to the amounts of ascorbic acid and of citrate-ion in 200 ml of orange juice. During the last 2 weeks, the low-milk group was given orange juice in place of the crystalline supplements.

The methods of collecting and preparing samples for analysis have been described by Metcalf and Hathaway ('45) and Watson et al. ('45). The nitrogen content of the foods and excreta was determined using the Hengar semi-micro Kjeldahl apparatus. The distillation tubes were equipped with water jackets (8 inches long and 3 inches in diameter) to cool the distillate and so prevent loss of ammonia.

RESULTS AND DISCUSSION

The data for this study are presented in table 1. Data for the preliminary adjustment periods (periods 1 and 4) have been omitted. A summary of the nitrogen balances in mg/kg of body weight is given in table 2.

Nitrogen intake, absorption, and retention. The protein allowance in the diets of these children was planned to meet the standards of Holt and Fales ('21) and was equal to or above the suggested allowances of the National Research

Average daily nitrogen balances for eight preschool children.

DIET	PERIOD	SUBJECT A INTAKE	SUBJECT A EXCRETION Urine	SUBJECT A EXCRETION Feces	SUBJECT A RETENTION	SUBJECT B INTAKE	SUBJECT B EXCRETION Urine	SUBJECT B EXCRETION Feces	SUBJECT B RETENTION	SUBJECT C INTAKE	SUBJECT C EXCRETION Urine	SUBJECT C EXCRETION Feces	SUBJECT C RETENTION	SUBJECT D INTAKE	SUBJECT D EXCRETION Urine	SUBJECT D EXCRETION Feces	SUBJECT D RETENTION
		mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
I [1]	5					8861	7762	969	130	8905	8212	766	—73	8790	7657	926	207
	6	9345	7659	883	803	9194	7400	891	903	9309	8100	800	409	9230	7488	874	868
	10	9188	7512	923	753	9118	7308	949	861	9188	7774	734	680	8949	7224	846	879
	11	9148	7874	800	474	8909	7425	889	595	9157	7896	709	522	8865	7228	734	903
	12	8762	7537	823	402	8567	7280	857	430	8735	7520	714	501	8567	7307	634	626
	Av.	9111	7646	857	608	8930	7435	911	584	9059	7900	745	415	8880	7381	803	697
II [2]	7	9033	7493	806	734	8873	7444	966	463	9033	7533	726	774	8829	7329	749	751
	8	9093	7436	891	766	9004	7339	883	782	9066	7601	752	713	8889	7502	554	833
	9	9002	7286	931	785	8923	7399	951	573	9020	7658	623	739	8816	7306	666	844
	13	8926	7605	777	544	8722	7456	883	383	8846	7525	694	627	8748	7354	583	811
	14	9020	7432	820	768	8719	7337	860	522	8869	7234	777	858	....	....	....	....
	Av.	9015	7450	845	719	8848	7395	909	545	8967	7510	714	742	8820	7373	638	810
III [3]	15	8866	7229	846	791	8707	7089	766	852	8804	7189	651	967	8751	7151	631	969
	16	8725	7243	789	693	8530	7048	923	559	8636	7372	651	613	8539	7020	551	968
	17	8583	7054	846	683	8397	7220	906	271	8548	7327	794	427	8432	7207	846	379
	Av.	8725	7175	827	722	8545	7119	865	561	8663	7296	699	669	8574	7126	676	772
IV [4]	18	8723	7197	826	700	8537	7006	906	625	8670	7255	674	741	8546	6927	703	916
	19	8789	7237	800	752	8630	7265	917	448	8701	7440	869	392	8639	7111	766	762
	20	8840	7316	789	735					8804	7404	757	643	8733	7220	646	867
	Av.	8784	7250	805	729	8583	7135	911	536	8725	7366	767	592	8639	7086	705	848
		SUBJECT E				SUBJECT F				SUBJECT G				SUBJECT H			
I [1]	7	8506	7299	777	430	8506	7785	331	390	8506	6960	950	596	7340	6029	797	514
	8	8560	7280	890	390	8700	7700	631	369	8704	6835	1049	820	7419	6090	844	485
	9	8594	7280	924	390	8748	7996	546	206	8752	7072	1056	624	7441	6190	769	482
	Av.	8553	7286	864	403	8651	7827	503	322	8654	6956	1018	680	7400	6103	803	494
II [2]	5	8800	7496	969	335	8848	8086	560	202	8871	7056	1084	731	7525	6114	951	460
	6	8728	7395	1070	263	8599	7929	546	124	8656	7059	1124	473	7382	6063	986	333
	10	8733	7210	917	606	8873	7793	506	574	8841	7041	1074	726	7595	6017	897	681
	11	8693	7344	876	473	8833	7801	506	526	8655	6779	1028	848	7548	5854	876	818
	12	8557	7198	854	505	8697	8006	563	128	8558	7071	1016	471	7541	6117	866	468
	Av.	8702	7329	937	436	8770	7923	536	311	8716	7001	1065	650	7500	6033	915	552
III [3]	13	8458	7706	820	—68	8598	7807	537	254	8343	6713	1011	619	7313	5991	911	411
	14	8532	7131	997	404	8667	7449	440	778	8358	6745	947	666	7386	5875	900	611
	15	8650	7282	857	511	8786	7606	569	611	8389	6755	894	740	7325	5975	800	550
	Av.	8547	7373	891	282	8684	7621	515	548	8363	6738	951	675	7341	5947	870	524
IV [4]	16	8626	7417	889	320	8770	7670	606	494	7208	6365	680	164	7359	6080	826	453
	17	8643	7347	847	449	8822	7594	574	654	7216	6135	631	450	7358	6009	849	500
	18	8604	7172	830	602	8773	7468	563	742	7165	6021	899	245	7288	5986	763	539
	Av.	8624	7312	855	457	8788	7577	581	630	7196	6173	737	286	7335	6025	813	497
V [5]	19	8633	7056	931	646	8756	7357	866	533	7199	5844	653	702	7257	5793	886	578
	20	8736	7103	894	739	8845	7132	871	842	7310	6151	644	515	7288	5852	737	699
	Av.	8684	7079	912	692	8800	7244	868	687	7254	5997	648	608	7272	5822	811	638

[1] Basal diet only. [2] Basal diet plus ascorbic acid. [3] Basal diet plus potassium citrate. [4] Basal diet plus ascorbic acid and potassium citrate. [5] Basal diet plus orange juice.

Council ('45), varying from 45 gm for the 3-year olds, to 58 gm for the 5-year olds.

Since chiefly meat and eggs were used to maintain the protein intake when the milk in the diet was decreased, the quality of the protein was not changed materially when the source was altered.

It is impossible to completely determine the availability of the protein in the diet, since there is no known analytical method for determining how much of the fecal nitrogen is of metabolic origin. The difference between the nitrogen intake and fecal nitrogen does give, however, a conservative estimate of the nitrogen available for body use. This may be considered as "absorbed" nitrogen, and in children usually represents approximately 90% of the ingested nitrogen (Macy, '42). Values for subjects A to H, respectively, were equivalent to 91, 90, 92, 92, 90, 94, 89, and 88% of the intake with standard deviations of $\pm$ 0.3% or less.

The average nitrogen retention values for the eight children of this study fall well within the range reported by Macy ('42), 700 $\pm$ 470 mg for 4-year olds, and 610 $\pm$ 390 mg for 5-year olds. Nitrogen intakes were similar for subjects A through F and for subject G on the first three diets, but the retentions varied widely, from — 73 to 969 mg per day for individual periods, or from 282 to 848 mg per day for the periods on a specific diet. Similar fluctuations have been reported by Macy et al. ('36). Although the intakes for subject H were lower than for the other children, the retention values were similar to theirs, ranging from 333 to 818 mg per day. After diet III, the basal food intake of subject G was cut, reducing the nitrogen intake about 1 gm per day. This lowering of the nitrogen intake reduced the nitrogen retention in the period immediately following the change, and the results for her will be omitted when data from diet IV are discussed.

According to Macy ('42) when reasonably constant amounts of high quality protein are ingested and absorbed, the level of protein intake is probably not the controlling factor in

nitrogen retention. The data for the children of this study support this view.

Effect of ascorbic acid supplement on nitrogen retention. Daniels and Everson ('37) found lower nitrogen retentions on a diet containing about 50 mg of ascorbic acid (including 30 mg of orange juice) than on larger intakes of ascorbic acid (part crystalline ascorbic acid and part from orange juice). Since other factors than ascorbic acid are added when orange juice is given, the increase in nitrogen retention should not be ascribed solely to the increase in ascorbic acid intake. In the subjects studied by Shepherd et al. ('40) the changes in nitrogen retention on the addition of 20 mg of ascorbic acid were not conclusive.

In the present study any effect of high levels of ascorbic acid on the nitrogen retention should be apparent in a comparison of the results obtained with diets I and II, and diets III and IV (table 1). The results were inconsistent: the nitrogen retentions were increased for subjects D and E and decreased for subject B, whether the ascorbic acid was added to the basal diet or to the basal diet plus potassium citrate. Values for subjects C and H were increased when the ascorbic acid was added to the basal diet, but decreased when it was added to the basal diet plus potassium citrate. Values for subject A were increased when ascorbic acid was added to the basal diet alone, but were practically unchanged when added to the basal diet plus potassium citrate; on the contrary those for subject F were increased only when the citrate was included. For any one child, comparison of the nitrogen retention values for all the periods when ascorbic acid was added (diets II and IV) with those when it was omitted (diets I and III) show no statistically significant differences.

From the results recorded above it appears that the addition of 100 mg of crystalline ascorbic acid to a basal diet containing about 25 mg of the vitamin does not consistently alter the nitrogen retention.

Effect of potassium citrate supplement on nitrogen retention. Increased retention of nitrogen was noted when the diet of infants was supplemented with salts of vegetable acids (Krause, '33) or with calcium citrate (Weber, '32). Davis ('35) found higher nitrogen retention on basic than on acidic diets by twelve children 7 to 12 years of age. Since the pH of the urines of the eight subjects in the present study was much more alkaline on the potassium citrate supplement, it seemed possible that the nitrogen retentions might have been increased by the addition of that salt. Individual variations in retention from period to period on the same supplement mask the effects of changes in supplement, so that variations because of addition of potassium citrate are not statistically significant for any child. The retentions for subject F, however, do seem notably higher with the supplement.

Effect of orange juice supplement on nitrogen retention. Approximately 218 ml of orange juice were added to the basal diet of subjects E, F, G, and H during the last 2 weeks of the study. The data for these periods are included in tables 1 and 2 under diet V. The average retentions of nitrogen on this supplement were the highest found during the study for subjects E, F, and H, and were higher for subject G than during any period on diet IV (basal diet supplemented with aascorbic acid plus potassium citrate), the only diet during which she received the same level of nitrogen intake. The percentage retentions of nitrogen were higher for all four children than on any other diet. These results confirm those of Shepherd et al. ('40) who found that fresh orange juice improved the nitrogen retention of young children to a greater extent than the addition of crystalline ascorbic acid and those of Chaney and Blunt ('25) who found increased nitrogen retention when orange juice was added to the diet of two older girls. They do not support the conclusion of Daniels and Everson ('37) that orange juice per se does not influence nitrogen retention.

Although the two periods during which orange juice was given in the present study are probably inadequate to estab-

TABLE 2

Summary of nitrogen balances of eight preschool children.

DIET	INTAKE	EXCRETION		RETENTION	
		Urine	Feces		
	mg/kg	*mg/kg*	*mg/kg*	*mg/kg*	*% of intake*
		Subject A			
I [1]	403	338	38	27	6.7
II [2]	399	330	37	32	8.0
III [3]	381	313	36	32	8.3
IV [4]	382	315	35	32	8.3
		Subject B			
I [1]	538	448	55	35	6 5
II [2]	533	445	55	33	6 2
III [3]	497	414	50	33	6 6
IV [4]	496	412	52	31	6.2
		Subject C			
I [1]	506	441	42	23	4.6
II [2]	493	413	39	41	8.3
III [3]	458	386	37	35	7.7
IV [4]	462	390	41	31	6.8
		Subject D			
I [1]	532	442	48	42	7.8
II [2]	519	434	38	48	9.2
III [3]	482	400	38	43	9.0
IV [4]	480	394	39	47	9.8
		Subject E			
I [1]	532	453	54	25	4.7
II [2]	538	453	58	27	5.0
III [3]	514	444	54	6	3 3
IV [4]	505	429	50	27	5 3
V [5]	503	410	53	40	8.0
		Subject F			
I [1]	466	422	27	17	3.7
II [2]	470	424	29	17	3.5
III [3]	454	398	27	29	6.3
IV [4]	447	386	30	32	7.2
V [5]	444	366	44	35	7.8
		Subject G			
I [1]	548	440	64	43	7.9
II [2]	548	440	67	41	7.5
III [3]	505	407	57	41	8.1
IV [4]	436	374	45	17	4.0
V [5]	438	362	39	37	8.4
		Subject H			
I [1]	535	441	58	36	6.7
II [2]	544	438	66	40	7.4
III [3]	521	422	62	37	7.1
IV [4]	511	420	56	35	6.8
V [5]	505	404	56	44	8.8

[1] Basal diet only.
[2] Basal diet plus ascorbic acid.
[3] Basal diet plus potassium citrate.
[4] Basal diet plus ascorbic acid and potassium citrate.
[5] Basal diet plus orange juice.

lish the effect of a supplement on the nitrogen retention, the results do indicate possible beneficial effects of orange juice.

SUMMARY AND CONCLUSIONS

Nitrogen metabolism has been studied in eight preschool children on protein intakes of between 45 and 58 gm per day. The basal diet was supplemented with ascorbic acid, potassium citrate, and orange juice as indicated. The results were as follows:

1. The nitrogen intake was probably not a factor influencing nitrogen retention, since from 88 to 94% of the ingested nitrogen was absorbed, and only 3.3 to 9.8% was retained.
2. The nitrogen retention was not significantly altered by the addition of 100 mg of ascorbic acid to a diet containing 23 to 25 mg of the vitamin.
3. The nitrogen retention was not significantly altered by the addition of 3.38 gm of potassium citrate to the diet.
4. The percentage retention of nitrogen was highest in all four subjects tested, when an orange juice supplement, equivalent in ascorbic acid and citrate value to the crystalline supplements, was added to the basal diet.

LITERATURE CITED

CHANEY, M. S., AND K. BLUNT 1925 The effect of orange juice on the calcium, phosphorus, magnesium and nitrogen retention and urinary organic acids of growing children. J. Biol. Chem., vol. 66, p. 829.

DANIELS, A. L., AND G. J. EVERSON 1937 The relation of ascorbic acid ingestion to mineral metabolism in children. J. Nutrition, vol. 14, p. 317.

DAVIS, N. J. 1935 Calcium, phosphorus and nitrogen retention of children. Effect of acid-forming and base-forming diets. Am. J. Dis. Child., vol. 49, p. 611.

HOLT, L. E., JR., AND H. L. FALES 1921 Food requirements of children. II. Protein requirement. Am. J. Dis. Child., vol. 22, p. 371.

KRAUSE, G. 1933 Langfristige Stoffwechseluntersuchungen an gesunden Säuglingen. Arch. f. Kinderh., vol. 99, p. 228.

MCKEY, B. V., M. F. CLARK, F. L. MEYER AND M. L. HATHAWAY 1946 Phosphorus metabolism of preschool children. J. Nutrition, vol. 31, p. 657.

MACY, I. G. 1942 Nutrition and Chemical Growth in Childhood. Vol. 1. Evaluation. Charles C. Thomas, Springfield, Ill.

MACY, I. G., H. A. HUNSCHER, F. C. HUMMEL, M. F. BATES AND M. W. POOLE 1936 Is the metabolic nitrogen balance an accurate measure for adequate protein consumption of children? J. Nutrition, vol. 11, suppl. p. 6.

METCALF, E. R., AND M. L. HATHAWAY 1945 Citrate metabolism of preschool children. J. Nutrition, vol. 29, p. 211.

MEYER, F. L. 1943 Ascorbic acid requirement and metabolism of preschool children. Thesis, Cornell University.

MEYER, F. L., AND M. L. HATHAWAY 1944 Further studies on the vitamin C metabolism of preschool children. J. Nutrition, vol. 28, p. 93.

NATIONAL RESEARCH COUNCIL 1945 Recommended dietary allowances. Bull. National Research Council, no. 122 (Aug.).

SHEPHERD, M. L., I. G. MACY, H. A. HUNSCHER AND F. C. HUMMEL 1940 Synthesized, processed and natural sources of vitamin C in the mineral metabolism of normal children. J. Ped., vol. 16, p. 704.

WATSON, E. K., E. W. MCGUIRE, F. L. MEYER AND M. L. HATHAWAY 1945 Calcium metabolism of preschool children. J. Nutrition, vol. 30, p. 259.

WEBER, G. 1932 Über die Einwirkung verschiedenen Salze auf den N-Stoffwechsel des Säuglings. Arch. f. Kinderh., vol. 97, p. 160.

THE COMPARATIVE GROWTH-PROMOTING VALUE OF THE PROTEINS OF WHEAT GERM, CORN GERM, AND OF SOME OTHER PROTEIN FOODS OF PLANT AND ANIMAL ORIGIN [1]

D. BREESE JONES AND KATHERINE D. WIDNESS
Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, U. S. Department of Agriculture, Washington

(Received for publication December 13, 1945)

Food products of plant origin have recently received particular consideration as sources of dietary protein that may be used to supplement or extend protein foods of animal origin in times of shortage. Outstanding among these are soybeans, peanuts, cottonseed, yeast, and corn and wheat germs. As compared with the others, the grain germs have received little attention with respect to the nutritional value of their proteins. Osborne and Mendel ('19) showed that wheat germ is more efficient for the growth and maintenance of rats than the whole wheat kernel. In a recent study, Hove and Harrel ('43a) concluded from the results of feeding experiments with young rats that "the protein of wheat germ as the sole source of protein in the diet is of as high a nutritional quality as animal proteins such as casein, skim-milk powder, dried egg white, and beef muscle." In a second paper these authors ('43b) reported that wheat germ and skim-milk powder are about equally efficient in improving the nutritive value of wheat flour, and excel corn germ and soybean meal in this respect. The superiority of wheat germ when

[1] Some of the data in this paper were presented in abstract form in Federation Proceedings, 1945, vol. 4, p. 156.

compared with corn germ as a supplement to wheat flour has been cited by Stare and Hegsted ('44). They also found that the proteins of wheat germ, corn germ, and skim-milk powder are practically of equal value in maintaining nitrogen balance in the adult dog. In nitrogen balance studies with growing rats, Mitchell and Beadles ('44) found that the biological value of the protein of corn germ is as high as that of round beef, and is 85% as digestible. Compared with autoclaved soybean protein, corn germ protein was found to be about equally digestible, but the digested protein was more available in satisfying the requirements of maintenance and growth.

The superior quality of the proteins of wheat and corn germs has been amply shown by the above cited investigations. It seemed desirable, however, to develop more information relating to their values as compared with those of other foods of plant and animal origin that are highly regarded as sources of dietary protein. Defatted wheat and corn germs, prepared for human consumption and having excellent keeping qualities, are now available in considerable quantities. It has been estimated that the potential annual production of wheat germ exceeds 150 million pounds, and that 600 million pounds of defatted corn germ could be produced.

EXPERIMENTAL

The growth-promoting values of the proteins of wheat germ and corn germ were determined by feeding experiments with young albino rats. For comparison, similar experiments were conducted on some other sources of high quality plant and animal proteins, namely, soybean, peanut, and cottonseed flours, skim-milk powder, dried whole egg powder, and casein (table 1). These materials supplied the sole source of protein in diets made adequate with respect to the essential nonprotein dietary factors. They were incorporated in the diets in quantities to supply protein at 10, 15, and 17.5% levels. The average net gain in weight of each group of animals over a period of 6 weeks and the average gain in weight per gm of

protein consumed were used as standards of comparison for evaluating the proteins of the materials studied.

All diets contained 2% cod liver oil and 4% salt mixture (Osborne and Mendel, '19). The remainder of each of the diets consisted of one of the protein sources listed in table 1, sufficient corn oil to adjust the total fat content of the diet to 8%, and sufficient dextrinized corn starch to adjust the protein content to 10, 15, or 17.5%. A vitamin mixture was incorpor-

TABLE 1

Composition of the protein foods.
The percentages are calculated on an air-dry basis.[1]

SOURCE	PROTEIN	FAT	SOURCE	PROTEIN	FAT
	%	%		%	%
Dried whole egg powder	46.00	34.27	Peanut flour	52.36	7.40
Skim-milk powder	37.31	....	Corn germ [2]	21.31	2.16
Soybean flour	46.73	6.47	Wheat germ [2]	34.30	0.40
Cottonseed flour	49.85	7.01	Casein [3]	89.70	...

[1] The following conversion factors were used in calculating the amounts of protein in the materials from their nitrogen content: Egg powder, 6.25; skim-milk powder, 6.38; soybean flour, 5.71; cottonseed flour, 5.30; peanut flour, 5.46; corn germ, 6.25; wheat germ, 5.80; casein, 6.38 (Jones, '31).

[2] Products defatted by solvent extraction at low temperature. Prepared by the Viobin Corporation, Monticello, Illinois.

[3] Labco.

ated in the dextrinized corn starch, which provided the following constituents in each 100 gm of diet: Thiamine hydrochloride, 0.2 mg; pyridoxine hydrochloride, 0.2 mg; riboflavin, 0.3 mg; calcium pantothenate, 0.3 mg; niacin, 1 mg; and choline hydrochloride, 10 mg. These vitamins were added to the dextrinized starch in an aqueous-alcoholic solution and the mixture was dried at 50°C. to the original weight of the starch.

The data presented represent results obtained with forty-nine lots of albino rats comparable in all respects. Forty-one of these lots were fed ad libitum and the remainder by the paired feeding method. Most of the lots consisted each of twelve animals (in some cases eight animals were used),

equally divided with respect to sex and having initial weights of 45 to 60 gm. They were housed in individual cages having wide mesh screen bottoms and kept in an air-conditioned room maintained at 76°F. (± 2°F.) and about 55% relative humidity. The animals on the ad libitum experiments were weighed and fed twice weekly. Fresh diets were prepared in kilogram quantities each week.

The paired feeding experiments were carried out as follows: Eight pairs of albino rats were used in each experiment. Each pair was of the same sex, from the same litter, and the pair mates did not differ in initial weight by more than 5 gm. The initial weights between pairs ranged from 45 to 60 gm. Both animals of a pair received a diet similar in all respects with the exception of the source of protein. One animal of a pair was fed ad libitum the diet that was consumed in the smaller quantity. The amount of food consumed by the other animal was determined by the quantity of food the rat fed ad libitum had consumed the previous day. In this way, the total food intake of both rats of a pair was practically the same at the end of the feeding period.

RESULTS AND DISCUSSION

As determined by the ad libitum method of feeding, wheat germ as a source of protein showed definitely higher nutritive values than corn germ at each of the three different levels of protein fed when calculated either on the net weight gains of the animals or on their gains per gm of protein consumed (table 2). This was especially pronounced at the 10% protein level.

The values of corn germ as compared with the oilseed flours varied with the different protein levels at which the materials were fed, when measured either by gains in body weight or by gains per gm of protein consumed. At a 10% protein level corn germ gave higher values than those similarly obtained with the oilseed flours when measured by gains in body weight. When measured in terms of weight gains per gm of protein consumed at a 10% level, soybean flour gave a higher value

than did corn germ. The comparative nutritive value of the plant protein studied at the 10% protein level on the basis of weight gains ranges from the highest downward in the following order: Wheat germ, corn germ, soybean flour, cottonseed flour, peanut flour. On the basis of gain in weight per gm of protein consumed they range in the same order,

TABLE 2

Comparative growth-promoting values of the proteins of wheat and corn germs, and of other protein foods, when fed as the sole source of protein in the diet. (Tests conducted by the ad libitum method of feeding over a period of 6 weeks.)

SOURCE OF PROTEIN	AVERAGE WEIGHT GAIN	STANDARD DEVIATION	AVERAGE GAIN/GM PROTEIN CONSUMED	STANDARD DEVIATION	AVFRAGE FOOD CONSUMED
	gm		*gm*		*gm*
		10% Protein in diet			
Wheat germ	132	± 29.12	2.54	± 0.190	518
Corn germ	101	± 15.83	2.11	± 0.178	478
Soybean flour	95	± 21.14	2.32	± 0.238	407
Cottonseed flour	82	± 12.38	1.89	± 0.162	431
Peanut flour	63	± 15.66	1.82	± 0.299	341
Whole egg powder	180	± 47.42	3.25	± 0.518	547
Skim-milk powder	130	± 15.87	2.64	± 0.170	492
Casein	120	± 14.68	2.41	± 0.225	497
		15% Protein in diet			
Wheat germ	140	± 34.19	1.84	± 0.201	504
Corn germ	114	± 30.61	1.56	± 0.245	481
Soybean flour	104	± 21.28	1.49	± 0.216	462
Cottonseed flour	132	± 20.44	1.80	± 0.137	489
Peanut flour	102	± 19.50	1.69	± 0.207	400
Whole egg powder	175	± 39.24	2.35	± 0.292	493
Skim-milk powder	161	± 37.86	2.05	± 0.234	518
Casein	135	± 35.61	1.74	± 0.241	510
		17.5% Protein in diet			
Wheat germ	131	± 35.67	1.58	± 0.251	467
Corn germ	112	± 23.71	1.29	± 0.171	491
Soybean flour	95	± 24.53	1.21	± 0.189	446
Cottonseed flour	132	± 20.96	1.64	± 0.182	460
Peanut flour	114	± 25.61	1.53	± 0.205	426
Whole egg powder	121	± 40.86	1.59	± 0.295	426
Skim-milk powder	144	± 26.98	1.82	± 0.203	452
Casein	174	± 27.65	1.90	± 0.231	524

with the exception of corn germ and soybean flour, which are reversed.

At the higher protein levels (15 and 17.5%) quite a different order of values was obtained. At the 15% level, wheat germ still held first place among the plant proteins both with respect to average weight gain and gain per gm of protein consumed. Cottonseed flour, however, ranked second. Corn germ came next in the order of values with respect to average weight gain, and soybean and peanut flours showed practically the same values. With respect to gain per gm of protein consumed peanut flour ranked third, with corn germ and soybean flour following as indicated in the table.

At the 17.5% protein level wheat germ and cottonseed flour gave practically the same values as indicated both by weight increases and gain per gm of protein consumed. Peanut flour, corn germ, and soybean flour followed in the order named.

When compared at the different protein levels in the diet, the animal proteins proved, in general, superior to the plant proteins. When fed at the 10% protein level dried egg powder gave the highest value obtained at any level with any of the proteins studied. At this level wheat germ and skim-milk powder gave essentially the same values with respect to weight gains and gains per gm of protein consumed, and both proved better than casein. With 15% protein in the diet, the highest values were obtained with egg powder. Skim-milk powder, wheat germ, and casein followed in the order named. At the 17.5% level casein gave the highest value. The low values found for the egg powder as compared with those obtained at the 10% and 15% protein levels are probably to be ascribed to a poorer quality product, the use of which was made necessary because of depletion of the original supply.

The values found for the corn germ and soybean flour proteins by the paired feeding method (table 3) are, in general, in fairly close agreement with those obtained by the ad libitum method (table 2). With wheat germ and dried egg powder, however, lower values were obtained by the paired feeding method. The nutritive values of the protein foods com-

pared, both with respect to body weight gains and gains per gm of protein consumed, were found in general to rank in the same descending order when determined by both procedures, namely, egg powder, wheat germ, corn germ, soybean flour. However, when measured by average gain in weight per gm of protein consumed the values for corn germ and soybean flour were essentially the same. In these experiments the materials studied were incorporated in the diets at a 10% protein level.

TABLE 3

Paired feeding tests.

Averages of results obtained with 8 pairs of rats used in each experiment. Ten per cent protein level in the diets. Duration of feeding tests — 6 weeks.

MATERIAL	AVERAGE BODY WEIGHTS		AVERAGE FOOD CONSUMPTION	AVERAGE	
	Initial	Final		Weight gains	Gain per gram of protein and standard deviation
	gm	*gm*	*gm*	*gm*	*gm*
Wheat germ	51	166	463	115	2.47 ± 0.175
Corn germ	53	154	465	101	2.18 ± 0.230
Wheat germ	53	172	442	119	2.69 ± 0.150
Soybean flour	55	150	430	95	2.20 ± 0.174
Corn germ	50	152	423	102	2.41 ± 0.207
Soybean flour	49	141	410	92	2.25 ± 0.237
Wheat germ	51	166	489	112	2.35 ± 0.253
Egg powder	52	194	490	142	2.91 ± 0.416

Hove and Harrel ('43b) reported that "a fair percentage of their rats fed wheat germ at 10% protein level in the diet developed a severe hemorrhagic disease characterized by sudden onset, marked weight loss, severe anemia, and in most cases death within four days." This difficulty did not occur when 1% of liver-extract powder was included in the ration. At none of the protein levels used in our rations was any adverse effect observed with either wheat germ or corn germ.

A statistical analysis was made to test the significance of the differences between the average weight gains obtained by the ad libitum method of feeding for the various protein sources. The weight gains for wheat germ and corn germ

were compared, first with one another, and then with each of the remaining proteins used in the experiment.

The highest degree of significance was found at the 10% level of protein in the diet. The differences in weight gain between wheat germ and all the other proteins, with the exception of skim-milk powder and casein, were found to be highly significant, while those for corn germ compared with the other sources were highly significant in all cases except soybean flour.

At the 15% level the significance of the differences was not so pronounced. A high degree of significance remained between weight gains for corn germ and those for whole egg and skim-milk powders, but the difference between the corn and wheat germs was only slightly significant. The differences between corn germ and the remaining proteins showed no significance. In the case of the wheat germ comparisons, the difference in gain from cottonseed flour was no longer significant, while peanut flour and soybean flour remained highly significant. Whole egg powder still showed a significant difference but to a lesser degree than at the 10% level.

Wheat germ was found to be significantly different only from soybean flour and casein at the 17.5% level, while corn germ differed significantly from cottonseed flour, skim-milk powder, and casein. The degree of significance for this level of protein was less in most cases than for the 10 and 15% levels.

It is of interest to note the downward slope of significance in the values with increasing protein levels in the diet above 10%.

SUMMARY

Growth-promoting values of the proteins of wheat germ and corn germ are reported. For comparison, values similarly obtained are given for several other protein foods of plant and animal origin — soybean, peanut, and cottonseed flours, dried whole egg powder, dried skim-milk powder, and casein.

The materials were fed as the sole source of protein to weanling rats at 10, 15, and 17.5% protein levels in diets approximately isocaloric and nutritionally adequate with respect to dietary factors other than protein. Both the ad libitum and paired feeding methods were used and the results were evaluated on the basis of weight increases and gains per gm of protein consumed over 6-week periods.

Consistently higher nutritional values were found for wheat germ than for corn germ at each of the different protein levels fed. Wheat germ also proved superior to the oilseed flours, and at the 10% level it was found as efficient as skim-milk powder, and more efficient than casein.

In general, the protein values of the materials determined by both the ad libitum and paired feeding methods were found to rank in the same descending order, namely, egg powder, wheat germ, corn germ, soybean flour.

Corn germ fed at 10 and 15% protein levels produced equal or greater weight gains than were obtained with peanut or soybean flours, but it was not as efficient as cottonseed flour when fed at 15 and 17.5% protein levels.

Definitely higher values at the 10 and 15% protein levels were obtained with the whole egg powder than with any of the other materials studied, but it was excelled by casein at the 17.5% protein level.

LITERATURE CITED

HOVE, E. L., AND C. G. HARREL 1943a The nutritive value of wheat germ protein. Cereal Chem., vol. 20, p 141.

———— 1943b Paper presented at the meeting of the American Association of Cereal Chemists, May, 1943. Unpublished.

JONES, D. B. 1931 Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. U.S. Dept. Agric. Circ. no. 183, 22 pp.

MITCHELL, H. H., AND J. R. BEADLES 1944 Corn germ: a valuable protein food. Science. vol. 99, p. 129.

OSBORNE, T. B., AND L. B. MENDEL 1919 The nutritive value of the wheat kernel and its milling products. J. Biol. Chem., vol. 37, p. 557.

STARE, F. J., AND D. M. HEGSTED 1944 The nutritive value of wheat germ, corn germ, and oat proteins. Fed. Proc., vol. 3, p. 120.

A STUDY OF PYRIDOXINE AND PANTOTHENIC ACID DEFICIENCIES IN THE MONKEY (MACACA MULATTA) [1]

KEITH B. McCALL, H. A. WAISMAN, C. A. ELVEHJEM AND EDITH S. JONES

Department of Biochemistry, College of Agriculture, and the Department of Bacteriology, Medical School, University of Wisconsin, Madison

THREE FIGURES

(Received for publication December 17, 1945)

Previous studies (Waisman and Elvehjem, '43; Waisman, Rasmussen, Elvehjem and Clark, '43) have demonstrated that the rhesus monkey (Macaca mulatta) grows normally and remains in good health when fed purified diets containing the known vitamins and a small amount of a liver fraction or a "folic acid" concentrate. Through the use of this diet specific vitamin deficiencies have been studied by withholding the vitamin concerned from the daily supplement (Waisman, '44; Waisman and McCall, '44; Waisman, McCall and Elvehjem, '45; Cooperman, Waisman, McCall and Elvehjem, '45).

The importance of pyridoxine and pantothenic acid in the nutrition of various laboratory animals has been studied by many workers. Wintrobe, Follis, Miller et al. ('43) have reviewed the early literature dealing with the production of pyridoxine deficiency and have presented data on the effects of vitamin B_6 deficiency in swine. These workers have

[1] Published with the appproval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by grants from the National Foundation for Infantile Paralysis and the University Research funds.

We are indebted to the Wilson Laboratories, Chicago, Illinois, for the liver products and to Merck and Co., Rahway, New Jersey, for the synthetic vitamins.

demonstrated a microcytic anemia and an increase of polychromatophiles, reticulocytes and nucleated red cells in the blood and a remission after the administration of vitamin B_6. Pantothenic acid deficiency has been produced in the mouse (György and Poling, '40), rat (Henderson et al., '42), chick (Waisman et al., '39), dog (Schaefer et al., '42), and pig (Wintrobe et al., '42) but the manifestations of the deficiency have been somewhat different in the various species. An account of the deficiency of pantothenic acid in swine has been reported by Wintrobe, Follis, Alcayaga et al. ('43). Impairment of growth, diarrhea, anorexia, unkempt hair, alopecia, and slight edema were the symptoms observed. Histological evidence demonstrated injury to the cells lining the glands of the colon. Blood studies have revealed a moderate normocytic anemia with a decrease in the plasma chlorides. Non-protein nitrogen increased only in extreme deficiency and may be due to renal failure.

In this paper we wish to describe pyridoxine and pantothenic acid deficiencies in monkeys as seen in this laboratory, the responses to synthetic pyridoxine and pantothenic acid, and the concurrent deficiency of the monkey anti-anemic factor found in liver, previously described by Cooperman, Waisman, McCall and Elvehjem ('45).

EXPERIMENTAL PROCEDURES

Young immature rhesus monkeys weighing approximately 1.5–2.0 K were used in all the studies. The methods of handling and feeding the animals have been described previously (Waisman, Rasmussen, Elvehjem and Clark, '43). The basal diet (M-3), consisting of sucrose 73 parts, purified casein 18, mineral salts 4, cod liver oil 3, corn oil 2, was fed ad libitum; and adequate quantities of ascorbic acid, thiamine, riboflavin, nicotinic acid, choline chloride, p-aminobenzoic acid, inositol, biotin, and pyridoxine or calcium pantothenate were given daily. In addition, a "folic acid" concentrate (Hutchings et al., '41) equivalent to 5 gm of solubilized liver powder (fraction L) was fed daily; this quantity was increased as the

experiments progressed to an equivalent of 10 gm of liver. The "folic acid" concentrate fed in the pantothenic acid studies was autoclaved at pH 10 for 30 minutes at 15 pounds pressure to destroy the pantothenic acid in the concentrate. Microbiological assays on the concentrate before and after this process showed that there was no loss of "folic acid" activity. After autoclaving, this material was cooled and neutralized before being fed.

The heated grain ration (241 H) of Kline et al. ('32) was also used for the production of pantothenic acid deficiency. It is low in pantothenic acid and is composed of yellow corn 58, wheat middlings 25, crude casein 12 parts which were mixed and heated for 30 hours at 120°C., salts IV 4, cod liver oil 3 and corn oil 2. It had been used extensively in the production of pantothenic acid deficiency in chicks (Waisman et al., '39) and rats (Henderson et al., '42), but had not previously been used for monkeys. This diet was supplemented daily with the same vitamin mixture that was used when the sucrose-casein basal (M-3) diet was fed.

Complete blood examinations including differential white cell counts (using Wright's stain) were made usually at weekly intervals throughout the period of depletion, deficiency and recovery. Hemoglobin was determined in the Evelyn photoelectric colorimeter and red and white cell counts were made in the usual manner by drawing blood from the marginal veins of the ear. Blood samples for chemical analysis were removed from the saphenous vein of the leg. The blood sugar, non-protein nitrogen and chlorides were determined on the Folin and Wu tungstic acid filtrates of the freshly drawn venous blood. Blood sugar was determined by the method of Benedict ('28), chlorides by the method of Whitehorn ('20–'21) and non-protein nitrogen by the method of Folin and Wu ('19).

PYRIDOXINE STUDIES

Four young monkeys (nos. 136, 137, 138, 139) were placed on the M-3 basal ration with the norite eluate concentrate plus all of the crystalline B vitamins except pyridoxine. All

four animals failed to grow normally and generally maintained their initial body weight with little variation for a period of about 9 months, when a marked loss of weight was observed in three of the animals. Test doses (1 mg per day) of pyridoxine were given for 7 days to two animals (nos. 136, 137) after they had been on this regimen for 6 months to determine if a pyridoxine deficiency was developing. Both animals showed immediate blood responses and weight gains

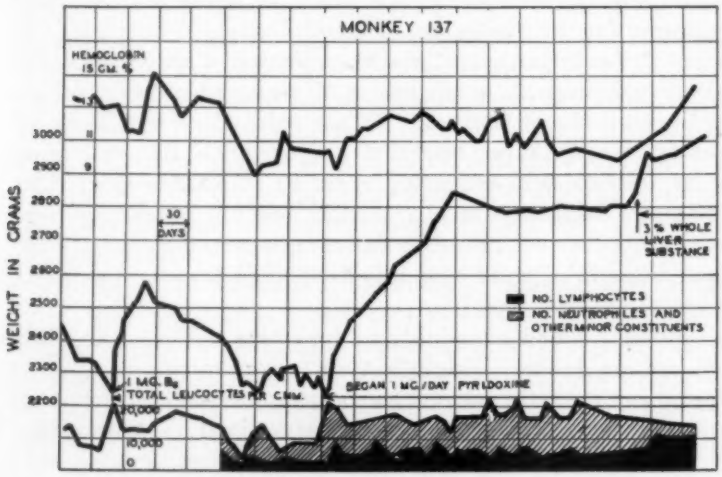


Fig. 1 Effect of pyridoxine deficiency on weight, hemoglobin, total and differential leucocyte count of a typical monkey, and the response to pyridoxine and whole liver substance.

as long as the pyridoxine was continued. The increased body weight was maintained for 3 months before it decreased to the same level as that before the administration of pyridoxine (fig. 1).

During this period of depletion, a hypochromic microcytic anemia developed in all of the animals and nucleated erythrocytes appeared in the blood. Hemoglobin values as low as 8 gm % were observed while the erythrocyte count decreased only slightly (4.5–5.5 million per mm^3). All four of

the animals at this time demonstrated marked inactivity, general weakness, hyperirritability and poor appetite, and had little interest in the vitamin supplement or the dry ration, although all of the supplement and a substantial amount of the ration were consumed during the day. All of the animals showed thinning of the fur and some greying of the fur on the legs, arms and back. However, the greying never progressed to a marked extent.

All four showed some polychromatophilia and this condition was more marked in two animals (nos. 136, 137). The total white cell count decreased, but the changes were so variable that no distinct pattern in the total count in relation to the anemia was discernible. However, the differential leucocyte count revealed an increase in the percentage of neutrophiles and a proportionate decrease in the percentage of lymphocytes. The neutrophiles increased to 55–60% of the total leucocytes, whereas the normal value is about 36% (Shukers et al., '38). At the same time the lymphocytes decreased from a normal of 59% to 25–35% of the total leucocytes. There was no significant change from the normal in the percentage of basophiles or eosinophiles and monocytes.

Within 2 weeks after 1 mg of pyridoxine per day was given to three of the monkeys (nos. 137, 138, 139), the animals began to gain weight at a normal rate, the hemoglobin values showed an increase and the polychromatophiles decreased. Nucleated red blood cells were no longer observed in blood. With the pyridoxine therapy the neutrophile-lymphocyte ratio tended to return to normal but the improvement was temporary and within 2 to 3 months the reversal was as marked as that preceding pyridoxine additions. Complete blood regeneration resulted within 5 weeks after whole liver substance was added at a level of 3% to the ration of 2 of these animals.

Monkey 136 was continued on the pyridoxine-deficient diet and slowly began to lose weight about 5 months after the other three animals first showed marked loss of weight. Since at this time the weight losses and anemia were not critical,

pyridoxine therapy (1 mg per day) was delayed for 3 months, during which time a rapid loss of weight occurred. Significant growth and hemoglobin responses occurred when pyridoxine was given, but the animal developed dysentery and died after 4 weeks.

It is significant that whole liver substances maintained the hemoglobin level at a value considerably higher than the published normal value of 12.2 gm % (Shukers et al., '38). These high values in the whole liver-treated monkeys, i.e., 14.5–15.5 gm % of hemoglobin, have been repeatedly observed in the monkeys in this laboratory which have received fresh or lyophilized liver (Cooperman, McCall and Elvehjem, '45) or whole liver substance.

PANTOTHENIC ACID STUDIES

Studies with the heated ration

The heated ration as described was fed to four monkeys (nos. 104, 105, 117, 118). Two of these (nos. 104, 105) served as controls and received 3 mg daily of calcium pantothenate. All of them grew slowly, but the two controls grew slightly better than the animals fed the heated ration with no added calcium pantothenate. After 6 months, it was observed that the deficient group showed poorer hair color, although the quantity of fur was apparently unaffected. Although the four animals were continued on this diet for 9 months, no significant differences in blood sugar, non-protein nitrogen, serum chlorides or serum proteins were observed. Since Schaefer et al. ('42) had reported hypoglycemia, increased non-protein nitrogen and a drop of serum chlorides in pantothenic acid-deficient dogs, it was apparent that pantothenic acid deficiency in the dog on the "synthetic diet" and in the monkey on the heated grain ration are not strictly comparable.

Studies with the synthetic ration

After 9 months the deficient animals (nos. 117, 118) were placed on the synthetic M-3 basal ration and given all the

B vitamins except calcium pantothenate. At the same time, five young monkeys were also placed on this regimen. The significant data for two of these monkeys (112 and 118) are given in figures 2 and 3 and a rather detailed description of the deficiency syndrome is included for monkey 112.

Monkey 112 ♂ (fig. 2). No increase in body weight occurred during the first 5 weeks and then the animal started to lose weight slowly without showing any gross symptoms.

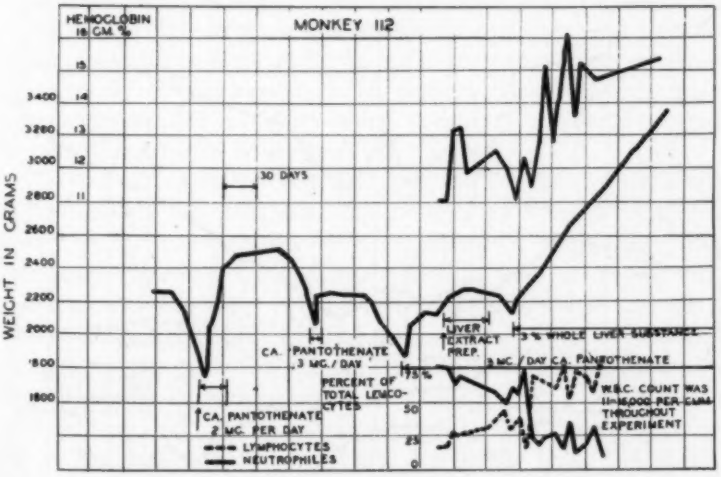


Fig. 2 Effect of pantothenic acid and liver factor deficiency on weight, hemoglobin, neutrophile and lymphocyte count of monkey no. 112 and the responses to calcium pantothenate, liver extract and whole liver substance.

After 4 weeks of continued loss in weight, 1 mg per day of calcium pantothenate was given and an immediate weight response resulted. This supplement was discontinued after 9 weeks. During the following 9-week period the animal gradually lost its fur, and became quite denuded and finally began to lose weight again. Following a weight loss of about 500 gm the biotin supplement was increased to 40 μg daily, but this change did not prevent a further weight loss. At this point 2 mg of calcium pantothenate per day were given and

continued for 16 days. Again a rapid weight response was obtained and new hair appeared after 2 weeks, although the new fur coat was not very heavy. Eight weeks after calcium pantothenate was discontinued the animal began to lose weight again and 3 mg of calcium pantothenate were given daily for 8 days. This treatment produced a weight gain, but during the 7 weeks following the removal of all the calcium pantothenate from the daily supplement, greying of the fur

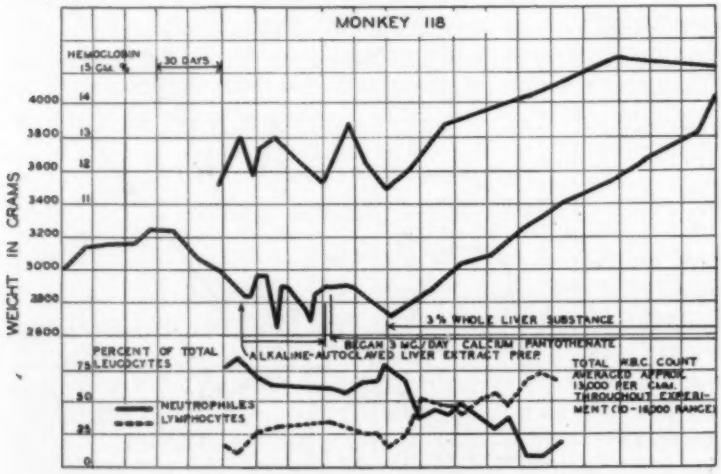


Fig. 3 Effect of pantothenic acid and liver factor deficiency on weight, hemoglobin, neutrophile and lymphocyte count of monkey no. 118 and the responses to a liver extract preparation, calcium pantothenate and whole liver substance.

and loss in weight resulted. The animal appeared emaciated, had unkempt fur, and showed evidence of muscular weakness in the legs. The blood picture indicated a mild anemia and a reversal of the neutrophile-lymphocyte ratio. The administration at this time of 3 mg of calcium pantothenate daily prevented further loss of body weight, but caused no general improvement in the condition; in fact, a large (3 cm) open sore developed on the bottom heel of the right foot and persisted for over 2 months in spite of treatment with

sulfathiazole applied topically. Monkey 122 developed similar sores on both feet that did not respond to either sulfathiazole or penicillin therapy.

After 3 months on this regimen, including 3 mg of calcium pantothenate daily, with no noticeable improvement, 3% whole liver substance was then added to the ration. Within 1 week the animal began to gain weight and became more active. The neutrophile-lymphocyte ratio returned to normal in 3 weeks and within 4 weeks the hemoglobin increased to 15.2 gm %. The animal was continued on this diet for 6½ months during which time the gain in weight was normal. Apparently a concurrent deficiency of factor(s) in whole liver required for complete blood regeneration also develops in pantothenic acid deficiency.

Monkey 118 ♂. Diarrhea and greying of the fur were observed about the time that the plateau in weight occurred. Four weeks later a pink rash appeared in the groin; this developed into an extensive dermatitis and scaliness and spread onto the inner areas of the calf and thigh and onto the inner side of the fore and upper arms. Dryness of the skin about the nose and unkempt fur were also seen. Definite improvement of the dermatitis occurred within 3 weeks after giving 3 mg per day of calcium pantothenate. At this time the ration was supplemented with 3% whole liver substance. Within 2 weeks, the monkey began to gain weight at a normal rate, the neutrophile-lymphocyte ratio and the hemoglobin returned to almost normal values. The whole liver substance was continued for 6 months and during this period the fur and the blood picture returned to normal.

In general the case histories of the other animals are similar to those described for monkeys 112 and 118.

DISCUSSION

It is evident from these experiments that monkeys given the purified basal ration with all the B-vitamins except pyridoxine develop a condition characterized by a hypochromic microcytic anemia, mild leucopenia, polychromatophilia,

presence of normoblasts, lack of growth, poor appetite and ataxia. In most respects these symptoms are similar to those observed in swine (Wintrobe, Follis, Miller et al., '43) and in dogs (McKibbin et al., '42).

Failure to produce a complete remission from the anemia with pyridoxine therapy has been observed both in dogs (McKibbin et al., '42) and in swine (Wintrobe, Follis, Miller et al., '43). A liver extract fraction (treated to remove the pyridoxine) was included in the ration fed to dogs, but these workers did not supply biotin, para-aminobenzoic acid or inositol. Smith et al. ('43) likewise have reported a typical hypochromic anemia in dogs which responded specifically to vitamin B_6 treatment, but brewers yeast at a level of 10% was required in addition to vitamin B_6 for complete blood regeneration.

Our results with pyridoxine deficient monkeys indicate that the additional factor is undoubtedly identical with the monkey anti-anemic factor required by riboflavin deficient monkeys to obtain complete blood regeneration. Differential white cell counts of riboflavin-deficient monkeys following the incomplete remission of the anemia by the administration of riboflavin reveal the same reversal of the neutrophile and lymphocyte count (unpublished data).

The pantothenic acid deficiency syndrome in monkeys is characterized by thinning and greying of the fur, lack of growth, extreme lassitude, emaciation, diarrhea, cachexia, ataxia and anemia. Follis and Wintrobe ('45) have demonstrated the role of pyridoxine and pantothenic acid deficiency in the degeneration of various portions of the nervous system (swine), and this action may account for the ataxia observed in our animals. The blood changes other than the severe anemia appear to be due to the concurrent deficiency of the factor(s) in whole liver substance. Monkey 117 that died on the pantothenic acid-low ration had a fatty and mottled liver and the adrenals were hemorrhagic, but no other gross pathological condition was observed.

It is significant that the blood picture which develops following calcium pantothenate therapy in the pantothenic acid deficient monkeys is identical with that which develops following incomplete remission of the anemia due to pyridoxine deficiency. Apparently this factor becomes limiting only after the pyridoxine or pantothenic acid is supplied following long depletion periods and with the resumption of growth. This would account for the short duration of the rapid growth responses following the administration of pyridoxine or pantothenic acid and the incomplete blood regeneration. Fresh or lyophilized liver (Cooperman, McCall and Elvehjem, '45) or whole liver substance supply the other factor(s) necessary to produce normal growth and concentration of blood constituents. Our data on the inactivity of 3% 1 : 20 liver extract powder in the prevention of this condition is further proof that the factor(s) is similar to that required to obtain complete blood regeneration and normal growth in riboflavin-deficient monkeys.

SUMMARY

Pyridoxine deficiency in young monkeys resulted when a synthetic diet was fed with all the crystalline vitamin B complex except pyridoxine. The symptoms were lack of growth, ataxia, hypochromic microcytic anemia, mild leucopenia, polychromatophilia and the appearance of nucleated red blood cells. With the administration of pyridoxine, growth was resumed and the anemia and blood picture improved.

A concomitant deficiency of the monkey anti-anemia factor occurred and was manifest by anemia and a reversal of the normal neutrophile/lymphocyte ratio. Whole liver substance at a level of 3% was necessary for optimum growth and blood regeneration.

Pantothenic acid deficiency syndrome was characterized by lack of growth, ataxia, greying and thinning of the fur, anemia, diarrhea and cachexia. Incomplete remission of these symptoms occurred on the administration of calcium panto-

thenate but complete recovery resulted when whole liver substance at a level of 3% of the ration was given.

LITERATURE CITED

BENEDICT, S. R. 1928 The determination of blood sugar II. J. Biol. Chem., vol. 76, p. 457.

COOPERMAN, J. M., K. B. McCALL AND C. A. ELVEHJEM 1945 Science, in press.

COOPERMAN, J. M., H. A. WAISMAN, K. B. McCALL AND C. A. ELVEHJEM 1945 Studies on the requirements of the monkey for riboflavin and a new factor found in liver. J. Nutrition, vol. 30, p. 45.

FOLIN, O., AND H. WU 1919 A system of blood analysis. J. Biol. Chem., vol. 38, p. 81.

FOLLIS, R. H., JR., AND M. M. WINTROBE 1945 A comparison of the effects of pyridoxine and pantothenic acid deficiencies on the nervous tissues of swine. J. Exp. Med., vol. 81, p. 539.

GYÖRGY, P., AND C. E. POLING 1940 Further experiments on nutritional achromotrichia in rats and mice. Proc. Soc. Exp. Biol. Med., vol. 45, p. 773.

HENDERSON, L. M., J. M. McINTIRE, H. A. WAISMAN AND C. A. ELVEHJEM 1942 Pantothenic acid in the nutrition of the rat. J. Nutrition, vol. 23, p. 47.

HUTCHINGS, B. L., N. BOHONOS AND W. H. PETERSON 1941 Growth factors for bacteria. XIII. Purification and properties of an eluate factor required by certain lactic acid bacteria. J. Biol. Chem., vol. 141, p. 521.

KLINE, O. L., J. A. KEENAN, C. A. ELVEHJEM AND E. B. HART 1932 The use of the chick in vitamin B_1 and B_2 studies. J. Biol. Chem., vol. 99, p. 295.

McKIBBIN, J. M., A. E. SCHAEFER, D. V. FROST AND C. A. ELVEHJEM 1942 Studies on anemia in dogs due to pyridoxine deficiency. J. Biol. Chem., vol. 142, p. 77.

SCHAEFER, A. E., J. M. McKIBBIN AND C. A. ELVEHJEM 1942 Pantothenic acid deficiency studies in dogs. J. Biol. Chem., vol. 143, p. 321.

SHUKERS, C. F., W. C. LANGSTON AND P. L. DAY 1938 The normal blood picture of the young rhesus monkey. Folia Hematologica, vol. 60, p. 416.

SMITH, S. G., R. CURRY AND H. HAWFIELD 1943 Vitamin B_6 deficiency anemia in the dog. Science, vol. 98, p. 520.

WAISMAN, H. A. 1944 Production of riboflavin deficiency in the monkey. Proc. Soc. Exp. Biol. Med., vol. 55, p. 69.

WAISMAN, H. A., AND C. A. ELVEHJEM 1943 The role of biotin and "folic acid" in the nutrition of the rhesus monkey. J. Nutrition, vol. 26, p. 361.

WAISMAN, H. A., AND K. B. McCALL 1944 A study of thiamine deficiency in the monkey (Macaca mulatta). Arch. Biochem., vol. 4, p. 265.

WAISMAN, H. A., K. B. McCALL AND C. A. ELVEHJEM 1945 Acute and chronic biotin deficiencies in the monkey (Macaca mulatta). J. Nutrition, vol. 29, p. 1.

WAISMAN, H. A., O. MICKELSEN AND C. A. ELVEHJEM 1939 The distribution of the chick antidermatitis factor (pantothenic acid) in meats and meat products. J. Nutrition, vol. 18, p. 247.

WAISMAN, H. A., A. F. RASMUSSEN, JR., C. A. ELVEHJEM AND P. F. CLARK 1943 Studies on the nutritional requirements of the rhesus monkey. J. Nutrition, vol. 26, p. 205.

WHITEHORN, J. C. 1920–1921 A system of blood analysis. Supplement II. Simplified method for the determination of chlorides in blood or plasma. J. Biol. Chem., vol. 45, p. 449.

WINTROBE, M. M., R. H. FOLLIS, JR., R. ALCAYAGA, M. PAULSON AND S. HUMPHREYS 1943 Pantothenic acid deficiency in swine with particular reference to the effects on growth and on the alimentary tract. Bull. Johns Hopkins Hosp., vol. 73, p. 313.

WINTROBE, M. M., R. H. FOLLIS, JR., M. H. MILLER, H. J. STEIN, R. ALCAYAGA, S. HUMPHREYS, A. SUKSTA AND G. E. CARTWRIGHT 1943 Pyridoxine deficiency in swine. Bull. Johns Hopkins Hosp., vol. 72, p. 1.

WINTROBE, M. M., M. H. MILLER, R. H. FOLLIS, JR., H. J. STEIN, C. MUSHATT AND S. HUMPHREYS 1942 Sensory neuron degeneration in pigs. IV. Protection afforded by calcium pantothenate and pyridoxine. J. Nutrition, vol. 24, p. 345.

COVITAMIN STUDIES

V. THE INTERRELATION OF ALPHA-TOCOPHEROL AND ESSENTIAL UNSATURATED FAT ACIDS [1]

E. L. HOVE AND PHILIP L. HARRIS

Research Laboratories, Distillation Products, Inc., Rochester, New York

THREE FIGURES

(Received for publication December 17, 1945)

The protection of vitamin A and carotene by tocopherols in vivo was the subject of the previous papers in this series (Hickman et al., '44; Harris et al., '44; Jensen et al., '43).

Destruction of carotene in the stomach of the rat requires the simultaneous presence of highly unsaturated fat acids. This was shown by Sherman ('41a) who also noted ('41b) that alpha-tocopherol prevented this destruction, in vivo. Peroxide formation at the double bonds of the fat acids probably occurs, with coupled oxidation (Sumner, '42) of carotene or vitamin A. Tocopherol protects the unsaturated bonds thus indirectly preserving the vitamin A. The oxidizing agent in the stomach responsible has been shown by Hove ('43) to be a water-soluble material similar to soybean lipoxidase.

Presumably a similar peroxidation of double bonds is responsible for the markedly increased vitamin E requirements when highly unsaturated fats are fed (Dam, '44; MacKenzie et al., '41).

If oxidation of the double bonds proceeds to a sufficient degree and extent, low levels of pure linolate should be less

[1] Communication No. 84 from the Laboratories of Distillation Products, Inc. A preliminary report of this work was published in Fed. Proc., vol. 4, March, 1945.

curative than the same levels with added α-tocopherol or other fat-soluble antioxidant for rats deficient in the essential unsaturated fat acids. This possibility has been tested and the results, which show that this is an actuality, are reported here.

EXPERIMENTAL

Diet and supplements

A basal fat-free diet, indicated as diet 60, was made up with the following percentage composition: vitamin-free casein [2] 20; sucrose 76; salt mixture (U.S.P. XI, No. 2, plus $ZnCO_3$, 0.1%; $CuSO_4 \cdot 7\,H_2O$, 0.08%; $MnSO_4$, 0.02%) 4. To this was added the following vitamins and related substances, in micrograms per gram of ration: thiamine hydrochloride 10, pyridoxine hydrochloride 10, riboflavin 10, calcium pantothenate 25, i-inositol 100, and choline chloride 1,000.

Vitamins A and D were fed weekly in the form of one drop (28 mg) of a fish oil concentrate, fortified with Delsterol, to furnish 2,000 units of A and 500 units of D per rat. In a few experiments crystalline carotene [3] and Delsterol were dissolved in coconut oil and one drop given daily to furnish 30 units of A and 10 units of D per rat. The synthetic water-soluble vitamins were thoroughly ground up with a small quantity of the casein, and this concentrate intimately mixed into the rest of the casein.

Methyl linolate was prepared every 10 days by the method indicated by Sherman ('41a). It was fed orally from a special capillary dropper calibrated to deliver exactly 5 mg per drop. The tocopherols were dissolved in propylene glycol and fed orally from droppers calibrated so that two drops were equivalent to 0.5 mg of the tocopherol.[4] The supplements were kept refrigerated in glass stoppered containers and flushed with nitrogen after each opening.

[2] Labco.

[3] Smaco.

[4] The alpha- and gamma-tocopherols used were the pure natural forms prepared by Dr. Meng in the Distillation Products Laboratories.

The sesame and olive oils used in some of the experiments were fresh samples of U.S.P. Grade. These were fed by droppers calibrated so that one drop was equivalent to 25 mg of the oils.

Essential fat acid deficiency in rats

Male albino weanling rats of the Sprague-Dawley strain were used. These were housed individually in an air-conditioned room maintained at 76°C. and 50% humidity. The rats were placed upon diet 60 at 40 to 45 gm body weight and maintained on this diet throughout the depletion period. When symptoms of the essential fat acid deficiency were evident the rats were divided into groups and started on curative experiments.

The criteria followed in these experiments were body weight, water consumption, severity of hind paw scaliness, and severity of caudal lesions. A discussion of these symptoms with respect to their use in assay procedures, and references to the original articles, has been given in a review by Burr ('42). Weekly records were kept of the symptoms.

Water consumption records were obtained by using covered cups with a 1-inch opening, wired to the side of the cage to prevent spillage. Weights of the cups plus water were taken at the beginning and end of a 24-hour period. A correction for water loss due to evaporation was obtained by use of identical cups in empty cages. This correction amounted to 3.0 to 3.5 gm. Usually the water consumption was determined on two adjacent 24-hour periods and the results averaged to give the daily water consumption for that week.

The severity of hind paw scale and caudal lesions was determined by weekly inspection of the rats. A numerical rating system, with 9 degrees, was used, ranging from "0" for freedom from symptoms, to "4" for maximum lesions, proceeding in "½" steps. In hind paw scaliness, the first stage ("½") was denoted by the first appearance of scale between the toes. The next major division was at the scale rating "2" when the scale had covered the top of the foot

and had appeared around the ankle. Stage "3" involved the first appearance of a slight dermatitis around the toes, and stage "4" represented this condition aggravated still further.

In judging caudal lesions the "½" stage indicated slight swelling and reddening of the tip (1 cm) of the tail, accompanied by slight circular ridging and the presence of a "fish scale" appearance for 1 or 2 cm above this. The condition progressed both in degree of the tail involved and in the severity of the lesions. Stage "2" involved the "fish scale" appearance over ⅔ the length of the tail, usually with necrosis setting in at the tip, and circular ridging fairly marked above this. Stage "3" was indicated by definite necrosis of the final inch of tail and marked progression of the circular ridging for ⅔ the length. In stage "4" the necrotic end had usually dropped off; the ridging had become acute with multiple open lesions and occasional bleeding. The tail was somewhat swollen, hard, and completely lacking in flexibility. The administration of curative agents reversed these steps.

The weekly numerical value for the lesion ratings of each group of rats on experiment was obtained by averaging the ratings of the individual members of the group.

In evaluating the results obtained it is felt that much more reliance can be placed upon growth and water consumption records, since these are obtained by physical measurements, than on lesion ratings which involve personal judgment. However, the ratings of the lesions do offer valuable corroborative evidence.

Rate of onset of fat acid deficiency as influenced by tocopherol

Sixty-two weanling rats were placed on diet 60. Eight were given 1 mg alpha-tocopherol daily, three were given 0.1 gm olive oil, equivalent to 6 mg linolate, and three were given both the olive oil and tocopherol supplements, daily. The remaining 48 were kept as controls. The average rates of change in body weights, water consumption, hind paw scale and caudal lesions are shown in figure 1.

Alpha-tocopherol, alone, had a slight beneficial effect on growth and delayed the onset of the typical lesions. This was especially true of the caudal lesions; up to 6 weeks on the depletion diet 50% of the rats receiving the tocopherol supplement had a caudal lesion rating of "½", the rest being still entirely normal. However, at this time 27% of the

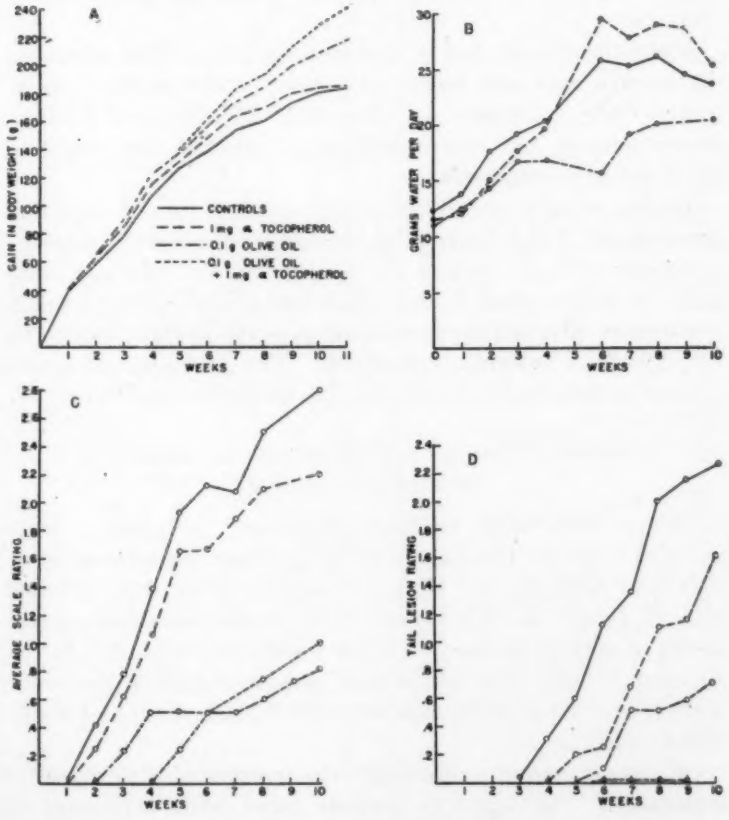


Fig. 1 Rate of depletion of essential unsaturated fat acid as influenced by tocopherol. The indicated supplements were fed daily. (A) Gain in body weight; (B) Average daily water consumption; (C) Average estimation of degree of scale on hind paw; (D) Average estimation of degree of caudal lesions.

control rats had severe tail lesions with ratings between "2" and "4" while only 19% were still normal. After 6 weeks on the depletion diet the rats receiving tocopherol rapidly began to develop the typical fat acid deficiency symptoms. This was especially noticeable in the water consumption records; the tocopherol-fed rats reached and maintained a higher water consumption level than the negative controls.

Alpha-tocopherol had a more marked beneficial effect on the growth rate and lesion prevention when given in addition to 6 mg of linolate (as olive oil). This level of linolate, alone, delayed, but was insufficient to prevent, the onset of the deficiency symptoms.

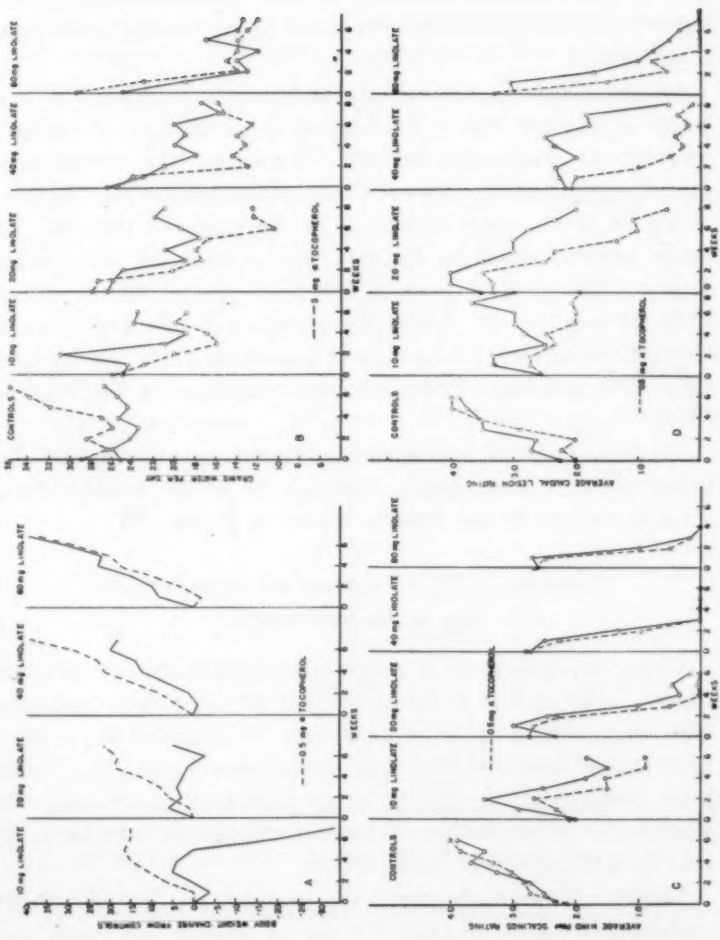
Similar results were obtained in another rate-of-depletion experiment. After 9 weeks on the depletion diet the average increases in body weight for groups of six rats receiving daily no supplement, 0.5 mg alpha-tocopherol, 10 mg linolate (as sesame oil), and the tocopherol plus the linolate, were 118, 127, 130, and 144 gm, respectively. The average tail lesion ratings at this time were 1.1, 0.5, 1.4, and 0.2, respectively.

Curative action of methyl linolate as influenced by alpha-tocopherol

Thirty rats with uniform deficiency symptoms were selected from the 48 controls of the previous experiment after they had been on diet 60 for 9 weeks. They were divided into 10 groups of three each. Five groups received supplements of methyl linolate six times weekly at levels of 0, 10, 20, 40, and 80 mg. The other five groups received the same methyl linolate supplements but with 0.5 mg of alpha-tocopherol in addition.

Figure 2 shows graphically the results of this curative experiment. The growth records have been expressed in

Fig. 2 Effect of tocopherol on the curative action of methyl linolate fed to essential fat acid deficient rats. The broken lines represent responses of rats fed 0.5 mg α-tocopherol daily, in addition to indicated linolate supplements.


CONTROLS
10 mg LINOLATE
20 mg LINOLATE
40 mg LINOLATE
80 mg LINOLATE
WEEKS
0·5 mg α TOCOPHEROL
A
B
C
D

terms of the body weight change of the supplemented animals as compared with that of the negative controls. The other criteria are expressed in terms of their absolute values. The control animals gained an average of 54 gm during the 8-week curative test.

Alpha-tocopherol improved the growth response to low levels of linolate, but at the highest level (80 mg) of linolate was of no additional benefit. Similarly, the alpha-tocopherol supplements increased the effectiveness of the low levels of linolate as measured by the rate of decrease of water consumption, or by the rate of cure of the caudal lesions. The rate of cure of the hind paw scaliness shows little if any benefit from the tocopherol. However, even without tocopherol, this symptom was cured very rapidly, and showed no grading of response with variation in the linolate level.

Alpha-tocopherol, alone, did not influence growth, but did bring about a noteworthy increase in water consumption. This is similar to the results shown in figure 1B.

Curative action of sesame oil as influenced by alpha-tocopherol

There appears to be a rough proportionality between the linoleic acid and tocopherol content of most fats and oils. However, sesame oil is an exception. It contains about 40% linoleic acid and less than 5 μg of tocopherol per gm. Therefore, sesame oil should be a good source of linolate for testing the interrelation of tocopherol and an essential fat acid in a triglyceride combination.

Thirty rats were depleted for 5 weeks on diet 60. Daily supplements of 0, 25, and 100 mg of sesame oil, with and without 0.5 mg of alpha-tocopherol, were started at this time. The group receiving each supplement was composed of six animals, except for the higher level of sesame oil, with and

without tocopherol. There were three animals in each of these two groups.

The influence of tocopherol on the curative action of sesame oil is shown in figure 3. The effectiveness of the lower level of sesame oil was clearly increased by the tocopherol sup-

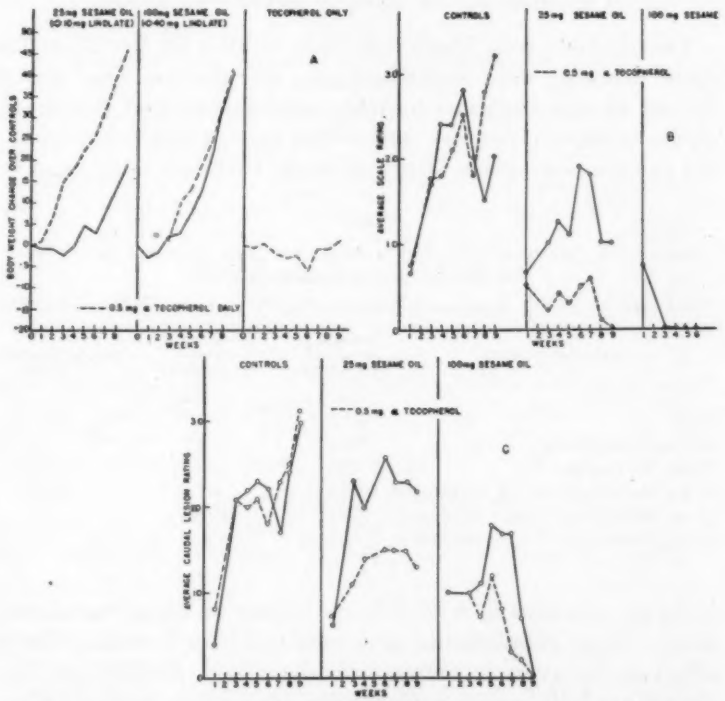


Fig. 3 Effect of tocopherol on the curative action of sesame oil fed to fat acid deficient rats. The broken lines represent responses of rats fed 0.5 mg α-tocopherol daily, in addition to the indicated sesame oil supplements.

plement, as is indicated by the growth, hind paw lesion, and caudal lesion charts. At the higher level of sesame oil this beneficial effect of tocopherol is not so evident. The control rats receiving no supplement gained an average of 79 gm during the 9 weeks' curative experiment.

Alpha-tocopherol, alone, had little influence on growth, but the rats in this group developed ear and nose lesions and a general unthrifty appearance not evident in the negative control group.

Variation of the level of alpha-tocopherol

Twenty-four rats which had been on diet 60 for 12 weeks since weaning were separated into six groups. One group served as the negative control, another received 0.5 mg of alpha-tocopherol per rat, daily. The rats of the four remaining groups were given 10 mg of methyl linolate daily, and in addition received 0, 0.25, 0.5, or 1.0 mg of alpha-tocopherol daily. These supplements were continued for 6 weeks. Their effect on the average change in body weight, and the average change in daily water consumption over this period, is given in table 1.

TABLE 1

Response of fat acid deficient rats to suboptimal doses of methyl linolate plus various levels of d, alpha-tocopherol.

(Four rats per group. Supplements started after 12 weeks on depletion diet 60.)

DAILY SUPPLEMENT	WEIGHT CHANGE IN 6 WEEKS	WATER INTAKE CHANGE IN 6 WEEKS	HIND PAW LESION CHANGE IN 6 WEEKS
	gm	*gm/day*	
None	17.7	+ 0.7	
0.5 mg α-tocopherol	14.0	+ 2.5	
10 mg Me-linolate	21.5	— 1.3	— 0.8
10 mg linolate + 0.25 mg α-tocopherol	55.0	— 7.2	— 1.2
10 mg linolate + 0.5 mg α-tocopherol	35.0	— 8.6	— 1.6
10 mg linolate + 1.0 mg α-tocopherol	52.3	— 11.0	— 1.0

The group receiving linolate alone gained an average of 3.8 gm more than the negative controls, while the average gain of the animals receiving linolate and 0.25 mg of tocopherol was 37.3 gm more. Larger amounts of tocopherol did not improve the growth performance. However, water consumption declined steadily as the tocopherol was increased to 1 mg daily.

Alpha- vs. gamma-tocopherol as sparing agents

It has been shown by Hickman, Kaley and Harris ('44) that gamma-tocopherol is equivalent to the alpha form as a sparing agent for vitamin A, in vivo. The relative potencies of these two compounds as sparing agents for methyl linolate

TABLE 2

The protection of suboptimal levels of methyl linolate in curing the fat acid deficiency.

(Six rats per group. Supplements started after 6 weeks — A, and 7 weeks — B, on diet 60.)

DAILY SUPPLEMENT	AVERAGE STARTING BODY WEIGHT		AVERAGE BODY WEIGHT INCREASE IN 5 WEEKS	
	gm	*Mean deviation*	*gm*	*Standard error*
A. Comparison of α- and γ-tocopherol				
None	156.5	± 19.2	30.7	± 2.3
0.5 mg α-tocopherol	169.6	± 10.5	28.3	± 1.9
20 mg linolate	154.9	± 7.7	50.7	± 2.6
20 mg linolate plus 0.5 mg α-tocopherol	159.8	± 14.0	65.7	± 3.2
20 mg linolate plus 0.5 mg γ-tocopherol	155.0	± 6.5	58.5	± 1.8
B. Effect of feeding on alternate days				
None	194.3	± 15.0	25.4	± 3.5
20 mg linolate daily	173.5	± 20.5	43.8	± 3.5
40 mg linolate fed every other day	183.8	± 9.5	45.5	± 3.4
20 mg linolate plus 0.5 mg α-tocopherol daily	186.0	± 9.7	51.8	± 2.3
40 mg linolate plus 0.5 mg α-tocopherol fed on alternate days	202.0	± 6.7	56.0	± 1.9

were tested. The results given in table 2-A indicate slightly less activity for the gamma-tocopherol. However, on statistical analysis the difference in response to the two tocopherols falls somewhat short of significance by the *t* test ($P = 0.05$).

The animals were depleted for 6 weeks on diet 60, after which supplements were begun, using six rats per group. After a 5-week supplementation period the group receiving 20 mg of linolate, alone, showed an average gain of 20.0 gm more than the controls. When alpha-tocopherol was given in addition, the growth gain averaged 35.0 gm. With gamma-tocopherol plus the linolate the growth gain averaged 27.8 gm.

Separate feeding of linolate and tocopherol

After 7 weeks' depletion on diet 60, rats were placed upon five supplemental variations using six animals per group. One group served as the negative control. Two other groups received, daily, 20 mg of methyl linolate; one of these was given 0.5 mg of alpha-tocopherol daily, in addition. The two remaining groups were given, every other day, 40 mg of methyl linolate; one of these groups received 1 mg of alpha-tocopherol on the days alternating with the linolate feeding. The experiment was continued for 5 weeks. The effect of these supplements on the growth of the animals is shown in table 2-B.

The average growth of the two groups receiving linolate was about the same. Alpha-tocopherol gave practically equivalent benefit whether fed daily with the linolate or on alternative days.

DISCUSSION

The data indicate that alpha-tocopherol extends the effectiveness of suboptimal quantities of linolate in preventing or curing the essential fat acid deficiency in rats. Gamma-tocopherol is also an effective sparing agent for low levels of linolate, but possibly less so than the alpha form.

The sparing action appears not to be limited to the gastrointestinal tract, since the onset of the deficiency syndrome was delayed by tocopherol given during the depletion period. Furthermore, it exerted a sparing action on low curative levels of linolate when fed separately at 24-hour intervals. This indicates a sparing of linolate in liver or tissue cells or

in body fluids, unless it is assumed that the tocopherol remained in the stomach for as long as 1 day.

When the deficient animal is flooded with large doses of linolate alone, rapid and complete cure results. Tocopherol fed in addition to these doses exerts no evident benefit on the rates of cure. Since additional tocopherol is not essential to the complete cure, the observed sparing of low levels of linolate must be due to a protective action, and is not simply the response to two independent deficiencies. It does not follow, necessarily, that because tocopherol gives no apparent benefit with sufficiently large doses of linolate, no interrelation exists between vitamin E and linolate in the ultimate biochemical function of the latter. Body tissue cells probably still contain fairly high levels of vitamin E even though the stores in liver and blood, needed for the in vivo sparing action, may be low.

Alpha-tocopherol given without linolate to rats which are suffering from an essential fat acid deficiency aggravates the symptoms. Two possible explanations are, either that the tocopherol acts as a prooxidant under these conditions and accelerates the destruction of the residual linolate, or that the tocopherol, acting as a physiological antioxidant blocks out more completely the normal and necessary oxidation of the residual linolate. The latter explanation implies that peroxidation at the central double bonds is involved in the essential biochemical function of linolate.

No proof exists for such peroxidation, in vivo, and there is evidence that complete central cleavage of the carbon chain does not occur. However, peroxidation may serve a function without leading to complete cleavage. Linolate peroxides may act as a potent terminal hydrogen acceptor for some chain of enzymatic oxidation, such as the β-oxidation of fats.

Salmon ('38) has noted that pyridoxine exerts a sparing action on essential fat acid. The structure of pyridoxine indicates that it should have antioxidant activity. To test this, freshly prepared free base pyridoxine was added at 0.08% to a vitamin A solution in fish oils. Incubation at 55°C.

showed that the stability of the vitamin A was doubled. This antioxidant activity of pyridoxine may be involved in the observed sparing of linolate, in vivo.

The significance to human nutrition of the sparing of linolate by tocopherol is complicated by lack of agreement concerning the essential nature of the unsaturated fat acids. There is evidence that infantile eczema is related to a deficiency in essential fat acids (Brown and Hansen, '37). The daily human requirement for linolate may be tentatively placed at 3 gm by calculating, on the caloric basis, from the rat's requirement of about 1 mg per calorie. Three grams of linolate would be contained in about one ounce of lard, or a quarter of an ounce of some vegetable oils. For efficient utilization of this quantity of linolate about 30 mg of tocopherol daily may be necessary. This calculation is based on the apparent ratio of 100 to 1 shown to give good protection in the rat.

SUMMARY

Alpha-tocopherol extends the effectiveness of suboptimal quantities of linolate in preventing or curing the essential fat acid deficiency syndrome in the rat. This sparing action has been shown using pure methyl linolate or sesame oil. Gamma-tocopherol also spares essential fat acid, in vivo.

The interrelation of tocopherol and linolate appears not to be restricted to the gastrointestinal tract, since feeding of these substances separately at 24-hour intervals still shows enhanced growth, as compared with either supplement by itself.

When tocopherol but no essential fat is fed to fat-deficient rats the deficiency symptoms are aggravated.

LITERATURE CITED

BROWN, W. R., AND A. E. HANSEN 1937 Arachidonic and linoleic acid of the serum in normal eczematous human subjects. Proc. Soc. Exp. Biol. Med., vol. 36, p. 113.

BURR, G. O. 1942 Significance of the essential fatty acids. Fed. Proc., vol. 1, p. 224.

DAM, H. 1944 Toxicity of fractions of hog liver fatty acids to chicks fed a vitamin E deficient diet. J. Nutrition, vol. 28, p. 297.

HARRIS, P. L., M. W. KALEY AND K. C. D. HICKMAN 1944 Covitamin studies II. The sparing action of natural tocopherol concentrates on carotene. J. Biol. Chem., vol. 152, p. 313.

HICKMAN, K. C. D., M. W. KALEY AND P. L. HARRIS 1944 Covitamin studies I. The sparing action of natural tocopherol concentrates on vitamin A. J. Biol. Chem., vol. 152, p. 303. III. The sparing equivalence of the tocopherols and mode of action. J. Biol. Chem., vol. 152, p. 321.

HOVE, E. L. 1943 The in-vitro destruction of carotene by water extracts of minced rat stomach. Science, vol. 98, p. 433.

JENSEN, J. L., K. C. D. HICKMAN AND P. L. HARRIS 1943 Effect of tocopherols and soybean phosphatides on utilization of carotene. Proc. Soc. Exp. Biol. Med., vol. 54, p. 294.

MACKENZIE, C. G., J. B. MACKENZIE AND E. V. MCCOLLUM 1941 The prevention by alpha tocopherol of "cod liver oil muscular dystrophy" in the rabbit. Science, vol. 94, p. 216.

SALMON, W. D. 1938 The effect of certain oils in alleviating localized erythematous dermatitis (vitamin B_6 deficiency) in rats. J. Biol. Chem., Proc., vol. 123, p. civ.

SHERMAN, W. C. 1941a The effect of certain fats and unsaturated fatty acids upon the utilization of carotene. J. Nutrition, vol. 22, p. 153.

———— 1941b Activity of alpha-tocopherol in preventing antagonisms between linoleic esters and carotene. Proc. Soc. Exp. Biol. Med., vol. 47, p. 199.

SUMNER, R. J. 1942 Lipid oxidase studies III. The relation between carotene oxidation and the enzymatic peroxidation of unsaturated fats. J. Biol. Chem., vol. 146, p. 215.

BIOLOGICAL VALUE OF PROTEINS IN RELATION TO THE ESSENTIAL AMINO ACIDS WHICH THEY CONTAIN [1]

III. COMPARISON OF PROTEINS WITH MIXTURES OF THE AMINO ACIDS

JOHN R. MURLIN, LESLIE E. EDWARDS, SERAPHINE FRIED AND THADDEUS A. SZYMANSKI
WITH THE ASSISTANCE OF
ELIZABETH NASSET, DORIS SMITH AND DOROTHY GILL
Department of Physiology and Vital Economics, University of Rochester, Rochester, New York

(Received for publication February 8, 1946)

That the essential amino acids must be determining of the biological value of proteins is so obvious as scarcely to require any introductory comment (Thomas, '30; Rose, '38). It is important now to learn (1) whether a mixture of the ten essentials in the proportion one to another contained in a protein can produce as much retention of nitrogen in relation to the amount absorbed, as can be obtained from the same amount of total nitrogen supplied by the protein itself, and (2) to what extent allowance must be made for the unnatural isomers of the racemic *(dl)* forms which cannot be utilized.

Before entirely artificial proteins are adopted for human nutrition — even emergency nutrition — it should be borne in mind that no proof has ever been given that all protein in the alimentary tract is broken down to the stage of biuret-free products before absorption (Van Slyke, '42). This being true it is possible that certain preformed aggregates (poly-

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Rochester.

peptids) are advantageous to protein nutrition. It is certain that entirely separate amino acids ingested in solution are absorbed more rapidly than natural products formed by proteolytic alimentary enzymes, and that a measurable portion escapes deamination, being consequently excreted unchanged in the urine. The question from this standpoint, therefore, is one of economy of utilization.

The supreme test of adequacy of a mixture of essential amino acids representing a protein as compared with the protein itself would be to feed the protein first, following a no-protein period, and immediately afterward a mixture of the ten in the same proportion one to another in which they occur in the protein and in sufficient quantity to supply the same total nitrogen as the protein. The amounts of the essentials over and above that represented by them in the protein should be sufficient to form all the nonessential amino acids supplied by the protein for whatever measure of usefulness they possess for the body. This was the hypothesis upon which the comparisons reported in this paper were based.

Obviously it was necessary first to learn the composition of the proteins selected with respect to the ten essentials. This analytical work will be reported in detail in a separate publication,[2] only such parts of it being used in this report as are necessary for clarity of presentation.

PROCEDURE

When this project was undertaken (August, 1943) only the racemic forms were available of isoleucine, leucine, methionine, phenylalanine, threonine, tryptophane and valine. For the basic amino acids arginine, histidine and lysine the *l* (+) monohydrochlorides were most available. All these[3] were

[2] A grant was obtained from the Nutrition Foundation and Dr. R. R. Sealock, at that time assistant professor in the department, agreed to supervise the analytical work. After Dr. Sealock left (January 1945) to become associate professor of biochemistry at Ames, Iowa, the responsibility was taken over by one of us (L.E.E.) and, when teaching duties interposed, it was passed along to Dr. Grant Bartlett, who served until the end of the project.

[3] Purchased from Merck and Company, Rahway, New Jersey.

confirmed as to purity by Kjeldahl determination of the nitrogen contents and as to freedom from heavy metals by spectrographic analysis very kindly performed for us by Dr. L. T. Steadman of the radiology department.

The source material of the protein having been analyzed, and the desired level of feeding, gauged by the urinary nitrogen excretion of the second or third day of no protein, having been decided, the test protein could be started; but the level of intake aften had to be adjusted after a day or two, when the "no-protein" fecal nitrogens became available. Only the last 2 or 3 days' urines of each period were used in the calculations. Because of unpredictable fecal losses from some of the food proteins these calculations involved appreciable error, but the aim was not to secure the most reliable biological values so much as to rate protein and amino acid mixture against the same basis of calculation. Paper I (Murlin et al., '46a) sets forth the issues which had to be dealt with here.

The analysis of a protein being in hand, the mixture of essential amino acids was compounded on the as-purchased basis to give as nearly as possible, taking into account anticipated larger urinary losses, a nitrogen balance at least as high as that of the protein. This mixture, fed in the period following the test protein, replaced it in the total diet (see paper I for the basal diet).

The amino acids were dissolved successively in distilled water in two or more 4-liter pyrex beakers over free flames, the temperature being kept below 80°C. to avoid discoloration of tryptophane. After 1 day's experience with each mixture the amount of water necessary could be estimated rather exactly. Pouring from one beaker to another was a decided help for obvious reasons, and as a rule the entire quantity daily for a squad of ten subjects could be dissolved in 6 or 7 liters at 80°C. The beakers then were partially cooled and their contents transferred, with liberal use of rinsing water to prevent crystallization, to 2-liter pyrex volumetric flasks and cooled to 20°C. The whole solution amounting to 8 or 10 liters was assembled in a 12-liter flask kept in the cooling water, and after thorough mixing each person's aliquot measured at 20° into bottles of appropriate size. Approximately one-third of the day's volume was consumed by sipping

frequently during each meal. The nitrogen content of the solution was checked by Kjeldahl analysis and almost always gave a result a little below the theoretical, even though the acids were kept in a desiccator.

The amino acid period varied from 3 to 6 days depending on how well the particular mixture was tolerated. If nausea was serious or vomiting occurred, the period was terminated at 3 days; if not it was continued to at least 4 days. Account always had to be taken of the remainder of the program for each series of experiments. If other "no-protein" and amino acid experiments were contemplated, it was not advisable to prolong the periods longer than 4 days because of the inevitable loss of weight during the amino acids regimes (see paper I, Murlin et al., '46a).

The subjects of these experiments were for the major part conscientious objectors assigned from their quota by O.S.R.D.

RESULTS

Seven of the ten experiments with amino acid mixtures listed in table 1 were performed in the order and manner described under Procedure and give the pattern characteristic of the regular routine. Three of them departed from the pattern in one or more respects. The first in the table had its "no-protein" period following those for the protein and amino acids. The second corn germ experiment (6–iii and iv) made use of an amino acid mixture to bring 19.44 gm wheat germ protein to an equality with 22.29 gm corn germ protein. The second experiment with egg protein (6–vii and viii) employed glutamic acid and glycine to make up the nitrogen of egg not supplied by essential amino acids. These three therefore are to be regarded as atypical experiments in method but equally instructive with the others in results.

To consider these first, the low biological value of egg protein in series 3 is accounted for in part by the reverse order of the protein and no-protein periods and in part by the high level of protein intake resulting in a plus N balance (Allison and Anderson, '45). The depleting effect of a no-protein period certainly promotes retention of a protein consumed immediately afterward. The experiment with wheat germ plus amino acids in comparison with corn germ showed that

TABLE 1

Synopsis of proteins and mixtures of essential amino acids consumed, nitrogen balances and biological values (B.V.).

SERIES NO.	PERIOD NO.	CONSUMED	NO. DAYS	NO. SUBJECTS	DAILY AVERAGES			
					Intake N	N bal.	B.V.	B.V. of amino acids as % of protein B.V.
3	viii	Whole egg	6	7	4.750	+ 0.374	75	
	ix	Amino acids	5	7	4.509	— 0.816	57	76
	x	No protein	3	7	0.333	— 2.853		
4	vi	No protein	3	11	0.292	— 3.411		
	vii	"Kitchen food yeast"[1]	5	11	3.700	— 1.003	83	
	viii	Amino acids	3	11	4.852	— 0.035	75	90
5	ii	No protein	3	10	0.361	— 3.481		
	iii	Cottonseed meal[2]	5	10	3.867	— 1.128	87	
	iv	Amino acids	3	10	4.353	— 0.599	74	85
	vii	No protein	3	7	0.324	— 3.038		
	viii	Corn germ	5	7	4.164	— 0.128	95	
	ix	Amino acids	5	7	4.247	— 0.123	83	87
6	ii	No protein	3	4	0.236	— 3.050		
	iii	Corn germ[3]	5	4	4.668	— 0.461	78.6	
	iv	Wheat germ[3] + Am. Ac. to equal corn germ	4	4	4.577	— 0.565	68.8	87
	vi	No protein	3	4	0.251	— 3.107		
	vii	Whole egg	5	4	3.305	— 0.184	101.7	
	viii	Amino acids, essential and nonessential	4	4	3.684	— 1.050	62.5	61
	x	No protein	3	2	0.433	— 3.029		
	xi	Haddock	5	2	3.245	— 0.685	92	
	xii	Amino acids	2	2	3.464	— 0.424	75	81
8	ii	No protein	4	9	0.806	— 3.471		
	iii	Corn germ	5	9	4.394	— 1.061	96.5	
	iv	Amino acids	4	9	4.195	— 1.409	66	68
	vii	No protein	4	9	0.526	— 2.769		
	ix	Corn germ	7	9	4.896	— 0.465	75.5	
	x	Amino acids adjusted	4	9	6.235	+ 0.023	54.7	74
	xii	No protein	4	8	0.195	— 2.842		
	xiii	Beefsteak	6	8	3.889	+ 0.484	91	
	xiv	Amino acids	3	8	3.983	— 0.473	67	74
		Amino acids adjusted	2	8	4.667	— 0.061	65	71

[1] Supplied by Anheuser-Busch Co. of St. Louis, Mo.

[2] Obtained from Oil Mill Products Co., Ft. Worth, Tex.

[3] Obtained from the VioBin Corp., Monticello, Ill.

by deducting the nitrogen of the unnatural isomers of the *dl* forms added to wheat germ the biological value of this protein could be brought into equality with that of corn germ (see footnote 9). The second experiment with egg makes it evident that neither glutamic acid nor glycine nor both aids retention of the total nitrogen represented by essentials and nonessentials.

It is clear from table 1 that none of the experiments with mixtures of essential amino acids compounded according to the analysis of the protein for these constituents, and supplying as much or more (three exceptions) nitrogen as the protein, produces a biological value so high as the protein itself when consumed by the same persons and evaluated against the same endogenous nitrogen. Expressed as a percentage of the B.V. of the protein, the B.V. of the corresponding mixture varied from 61% (series 6, period viii, essentials and nonessentials) to 90% (4–viii, amino acids containing nitrogen enough to approach equilibrium closely). There is no regular pattern of performance on the amino acid diet as compared with that on protein. This was scarcely to be expected.

The failure of such mixtures to duplicate the biological values of the proteins, which they imitated with respect to these ten constituents more or less exactly, was not surprising in view of the facts developed by Rose and co-workers (Rose, '38), Totter and Berg ('39), Berg ('42), Bauer and Berg ('43), Albanese ('44) and others regarding the nutritional inequality of the *d* and *l* isomers. According to the reviews by Rose and Berg the unnatural isomers of isoleucine, leucine, valine and threonine are not utilized at all for maintenance and growth in rats, and according to Bauer and Berg they are also not available to the mouse. Totter and Berg ('39) reported that the unnatural isomers of tryptophane and histidine were not so effective in the mouse as they have been found to be for the rat, and that unnatural lysine is not effective at all in either species. While Bauer and Berg found both optical isomers of phenylalanine and methionine apparently equally well utilized for growth in mice (as they are in

the rat), Albanese believes on the basis of his excretion studies that the unnatural isomers of tryptophane and phenylalanine are not readily utilized (retained) by man. Since differences have been found between two species so closely related as the rat and mouse with respect to the utilization of unnatural tryptophane, histidine and possibly leucine, Albanese expresses the view that "it is obviously unsafe to make assumptions about man on the basis of animal studies."[4] However, accepting the results on rats and mice as at least indicative of what may be expected in maintenance metabolism in man, and the scanty evidence obtained directly from human subjects, it appears that there are six *dl* forms, isoleucine, leucine phenylalanine, threonine, tryptophane and valine, whose unnatural isomers may be rejected by man. Hence it does not appear at all gratuitous to assume that under the conditions of these experiments when sufficient amounts of the natural isomers are supplied the unnatural ones should be regarded as completely dispensable.

The problem then was, first, to determine whether an overall correction could be made in the mixture to compensate for this inequality. If it were certain that all of the nitrogen of the unnatural form in the *dl* isomers were wasted without being deaminated, it would be justifiable to deduct the total amount of this nitrogen from the absorbed and urinary nitrogens and recalculate the biological value on the remainder absorbed and excreted. This is regularly done in this laboratory for the purine nitrogen of coffee, tea and carbonated beverages, because they are largely excreted unchanged in the urine. The same treatment would be proper for medicinal nitrogen or for that of urea added to the diet as a test substance for kidney function. Rose ('38, p. 131) states it is customary in his laboratory and elsewhere "if a synthetic product is employed to double the amount in order to insure the presence of the active isomer at the desired level." This procedure assumes that none of the unnatural isomer is util-

[4] One is reminded of Mitchell's ('38) comment: "It is good that our complacency in the infallibility of a sort of Jeffersonian doctrine that all animals are created equal is being disturbed."

ized for retention and has been tried several times in this study, but with no better results, as will be seen in table 2. Without correction the B.V. is too low; with correction for all the nitrogen of the unnatural isomers it is too high.

All three of the derived values in the examples given overcorrect the B.V. of the proteins. Likewise all the other experiments in table 1 revealed overcorrection when all the nitrogen of the unnatural forms was deducted. This obviously means that some of it, but a varying proportion from experiment to experiment, was utilized in retention, therefore presumably in true anabolic processes. From present knowledge

TABLE 2

Theoretical correction for N of unnatural isomers in experiments with egg and yeast proteins.
(All values are average for entire squad.)

	a	b	c	d	e	f	g	h	i	j	k
	Test protein fecal N	No-protein fecal N	(a–b) Fecal waste N	Test protein N eaten	(d–c) Absorbed N	True digestibility %	Test protein urine N	No-protein urine N	(g–h) urinary waste N	$\frac{i \times 100}{e}$	(100–j) B.V.
Egg											
(3–viii)	0.970	0.822	0.148	4.750	4.602	97	3.406	2.350	1.156	25.0	75
Am. Ac.											
(3–ix)	1.192	0.822	0.370	4.510	4.140	92	4.133	2.350	1.783	43.0	57
					1.289		1.289				
		Correction for all N of unnatural isomers:			2.851		2.844	2.350	0.494	17.3	82.7[1]
Yeast											
(4–vii)	1.544	1.074	0.470	3.700	3.230	86	3.159	2.629	0.530	16.4	83.6
Am. Ac.											
(4–viii)	0.773	1.074	— 0.301	3.772	4.073	108	3.653	2.629	1.024	25.1	74.9
					1.046		1.046				
		Correction for all N of unnatural isomers:			3.027		2.607	2.629	— 0.022	0.07	100.1[1]
Am. Ac.											
(4–viii)	0.773	1.074	— 0.301	4.852	5.153	94	4.116	2.629	1.487	28.9	71.1
doubling *dl* forms					1.365		1.365				
		Correction for all N of unnatural isomers:			3.788		2.751	2.629	0.122	3.5	96.5[1]

[1] Overcorrected.

it is not possible to deduce from the literature a single correction factor to compensate exactly for the nutritional inequality in the case of each of the seven racemic forms used in this study.

The literature does, however, clearly reveal that the unnatural isomers are the ones, which, though they may be made to fit the body needs in emergency perhaps after acetylation (duVigneaud, Sealock and Van Etten, '32; Holt and Albanese, '45), either like foreign or medicinal substances or like nonessential surplus amino acids, by oxidative deamination. If any unnatural isomer should be, like ingested urea, wholly useless, it would be found in the amino acid fraction of the urinary nitrogen. If like nonessential amino acids it should be deaminated and transformed into urea or ammonia it would be found in one or the other of these fractions. Can we not then change the level of feeding so that, assuming the same rate of performance of the human organs, the whole of the nitrogen of these scarcely assimilable intruders would be rejected in one form or another?

It is not difficult to calculate how much nitrogen would need to be subtracted from the absorbed and urinary nitrogens in any experiment with the amino acids to give the correct biological value. The formula is the following:

$$(1)\quad \text{B.V. of the Protein as percentage} = \frac{\left|\ \text{Retained N}\ \right|}{\text{Absorbed N-x}} \times 100$$

$$\left|\ [(\text{Abs.N-x}) - ((\text{Ur.N-x}) - \text{No-Prot.Ur.N})]\ \right|$$

x will be something less than the total nitrogen of the unnatural isomers fed. Suppose it were two-thirds of the total. This would imply that one-third of the nitrogen of the unnatural forms already is being excreted. If so, increase of the *dl* isomers by two-thirds should permit excretion of the total nitrogen of these forms as "medicinal" or useless nitrogen, because then the nitrogen of the natural forms would be sufficient, having also been increased by two-thirds.

An experiment performed late in the course of the investigation illustrates just such a correction. At the time the

analyses of the hydrolysates for the essential amino acids were more satisfactory, because confirmed by high precentage recoveries of pure amino acids added singly to the hydrolysates. Likewise the fourth-day no-protein urinary nitrogen excretions were dependable as endogenous base according to the criteria developed in paper I (Murlin et al., '46a). The experiment referred to will be found in series 8, periods ix and x, of table 1.

Corn germ protein and its amino acid counterpart

The series started with a new squad of nine men. Corn germ was again chosen as source of the test protein. A preliminary experiment on this protein followed by the corresponding mixture of the ten essential amino acids had been run for the express purpose of deriving a correction factor for the nitrogen of all the unnatural isomers taken together, which could be applied in a repetition of the experiment. Urinary nitrogens of the first no-protein period (8–ii) were not yet low enough for a dependable absolute biological value; but it was decided, since the men were scheduled for at least two more such periods, to accept a high B.V., because whatever its level a correction derived by comparison of protein and amino acid mixture referred to the same base probably should apply satisfactorily later against true endogenous excretions.

The calculation by means of equation (1) above produced a value for x very close to two-thirds (66.9%)[5] of the total nitrogen of the unnatural isomers fed and, as it happened, just 33.3% of the total average amino acid nitrogen fed in the preliminary experiment; namely, 3.977 gm daily. The nitrogen balances were too low as may be seen in table 1 (8–iii and iv), to protect body protein. It was desirable from this point of view also to increase the nitrogen intake. Allison and Anderson ('45) have shown in careful experiments on dogs that B.V. is a constant so long as absorbed nitrogen and nitrogen balance from the same protein bear a linear relation-

[5] On recalculation an error was found, correction of which made the percentage 60.3%, but it was then too late to change.

ship to one another, but this applies only to the region of negative balances. Their values are expressed in terms of surface area. But whatever the mode of expression it is evident that where there is excessive excretion, as in these experiments, due to the more rapid absorption of amino acids than of products of protein digestion and to the relatively lower nutritional value of at least most of the unnatural isomers, if not all of them, no such relationship could be expected, between protein and amino acid mixture.

The corn germ feeding was increased by 0.75 gm nitrogen (average per man) and produced a B.V. of 75.5 (table 3) (Mitchell and Beadles, '44). Increasing the *dl* amino acids by 66.9% above the level calculated to equal the nitrogen intake on corn germ and maintaining the percentages one to another of these seven constituents produced the B.V. of 54.7. The three basic amino acids were held at the calculated level before increasing the *dl* forms, and as a consequence the total nitrogen of the unnatural isomers now amounted to 28% of the total nitrogen of all the amino acids, 5.982 gm, instead of 33.3% for the preliminary experiment. Applying

TABLE 3

Biological value of corn germ protein (period 8–ix, table 1).

c Fecal N correction	d Test protein N fed	e (d–c) absorbed N	f True digestibility	g Test protein urine N	h No-protein urine N	i (g–h)	j $\frac{i \times 100}{e}$	k 100–j B.V.	N-bal.
1.147	4.896	3.749	77	3.133	2.215	0.918	24.5	75.5	— 0.465
Biological value of amino acid mixture with *dl* forms increased by 66.9% (period 8–x, table 1).									
0.166	6.235	6.069	97	4.966	2.215	2.751	45.3	54.7	+ 0.023
Correction for all N of unnatural isomers fed:		1.675		1.675					
		4.394		3.291	2.215	1.076	24.5	75.5	+ 0.023
Correction for excess of urea, NH_3 and Am. acid N in urine:		6.069		4.966					
		1.712		1.712					
		4.357		3.254	2.215	1.039	23.8	74.2	+ 0.023

this correction on the assumption that all of this nitrogen of the unnatural isomers is now dispensable, the B.V. of the protein is reproduced precisely.

This experiment falls short of perfection, however, for the proper correction factor was not used (see footnote 5). But if the factor 60.3% had been used, the effect on B.V. would have been very small.[6] A further check on the correction for a fraction of the total nitrogen equal to that of the unnatural isomers, is found by taking into account the distribution of nitrogen in the urine, as will be explained more fully in connection with the beefsteak experiment. The extra urea and ammonia N deriving from the adjusted amino acid intake was estimated at 1.299 gm and the extra amino acid nitrogen equal to 0.483 gm, a total of 1.712 gm. Deducting 1.712 gm, in place of the 1.675 gm representing the total nitrogen of the unnatural isomers, produces a B.V. of 74.2 (table 3). There is of course no way of proving that all the extra urea, ammonia and amino acid nitrogen[7] came from the unnatural isomers, but it is at least a striking coincidence that the sum of these fractions so nearly equalled just one-half of the total nitrogen of the *dl* isomers at the adjusted level.

Nitrogen balances of protein and its amino acid counterpart need not be equal to give equal biological values (table 3); but it is important that the nitrogens absorbed be at least close together and the correction accomplishes this with reference to the isomers of the amino acids known to be available for synthesis.

This demonstration creates a strong probability, though not direct proof, that when sufficient amounts of natural isomers are present the unnatural forms become entirely dispensable.

[6] Thus the total N of the unnatural isomers would have been 27.5% of the total amino acid nitrogen, the correction 1.604 gm instead of the 1.675 gm used in table 3, and the B.V. 73.5. This assumes that the urinary excretion had remained the same. It could not have been higher, and if it had been reduced, the limit would have been 0.132 gm; consequently the B.V. would have fallen between 73.5 and 76.7.

[7] A few tests performed by Dr. Grant Bartlett, employing a *d*-amino acid oxidase, prepared from acetone powders of rabbit kidney and liver, estimated the *d*-amino acid nitrogen on 2 days of this period at 17 to 21% of the total amino acid nitrogen in the urine or about 10.4% of the nitrogen of the unnatural isomers fed.

Such a result could not have been obtained if the excretion data had not been entirely reliable.

Another and perhaps more pertinent criticism would be that by increasing the *dl* forms alone, leaving the basic amino acids unchanged, the proportions of the latter necessarily were reduced and the former increased to such an extent that the resulting mixture no longer duplicated the composition of the corn germ protein. A look at the two mixtures before and after adjustment of the *dl* forms shows how much they were changed (table 4).

TABLE 4

Distribution of the essential amino acids in corn germ protein and in the mixture of synthetic amino acids (on the as-purchased basis, containing 66.9% increase of dl forms).

CORN GERM PROTEIN [1]		AMINO ACID MIXTURE [2]		
	%			%
Arginine	16.19	*l* (+)	Arginine	12.88
Histidine	5.87	*l* (+)	Histidine	5.17
Isoleucine	6.48	*dl*	Isoleucine	7.04
Leucine	18.94	*dl*	Leucine	20.57
Lysine	12.28	*l* (+)	Lysine	10.99
Methionine	7.83	*dl*	Methionine	8.51
Phenylalanine	9.52	*dl*	Phenylalanine	10.34
Threonine	9.16	*dl*	Threonine	9.95
Tryptophane	6.11	*dl*	Tryptophane	6.46
Valine	7.44	*dl*	Valine	8.09
	99.33			100.00

[1] The essential amino acids make up 62.19% of the total protein (N × 5.7) of corn germ. The percentages given reveal the proportion of each to all and to one another in this 62.19%.

[2] This is the adjusted or final mixture.

Comparison of the figures in table 4 makes evident that all the basic amino acids are reduced more than the *dl* forms are increased, the relative change of the former being from 10.5% for lysine to 19.8% for arginine; for the latter from 8.4% for leucine to 8.8% for valine.

Calculated on the percentage of the nitrogen of the *l* or natural forms, which is more important from the point of view of our working hypothesis, the change, as seen in table 5 (cols. 3 and 5) is found to be reversed. All of the basic amino acids

now are increased because all of their nitrogen is of the natural form, while all the nitrogens of the *l* isomers are lower than in the protein, because obviously only half of the nitrogen of the *dl* isomers is considered. The relative change is now 8.4% for lysine, 8% for histidine and 5% for arginine. The relative decrease among the *dl* forms runs from 9 to 11%. On the evidence in table 3 it is believed these alterations are not significant for biological values.

TABLE 5

Distribution of nitrogen of the natural isomers among the essential amino acids of corn germ protein and the mixture of synthetic amino acids.

	CORN GERM PROTEIN			AMINO ACID MIXTURE		
	Gm N/ 100 gm	Per cent of total N	Av. N Gm/man/day essentials only	Gm N/ 100 gm	Per cent of total N	Av. N Gm/man/ day
Arginine	3.27	32.3	0.932	3.27	34.9	1.502
Histidine	0.99	9.8	0.292	0.99	10.6	0.456
Isoleucine	0.43	4.2	0.121	0.36	3.8	0.166
Leucine	1.26	12.5	0.361	1.05	11.2	0.483
Lysine	1.46	14.4	0.418	1.46	15.6	0.674
Methionine	0.46	4.5	0.130	0.38	4.0	0.172
Phenylalanine	0.50	4.9	0.141	0.41	4.4	0.188
Threonine	0.67	6.6	0.190	0.56	6.0	0.257
Tryptophane	0.52	5.1	0.147	0.43	4.6	0.198
Valine	0.55	5.4	0.156	0.46	4.9	0.211
Totals	10.11	99.7	2.888	9.37	100.0	4.307
	Total N of the unnatural isomers of the 7 racemic forms 1.675					

The essential amino acids in corn germ protein (N×5.7) according to the analysis made in this laboratory accounted for only 62.2% of the total nitrogen, and the nitrogen of these natural acids in the average amount ingested by the squad daily was only 2.888 gm while the nitrogen of the natural isomers in the average daily ingestion of the amino acid mixture was 4.307 gm. The partial contribution of each acid in the two diets is found in columns 4 and 7 of table 5. The percentage relationship of the nitrogens of the essential amino acids one to another evidently is not so different in the two forms of feeding the natural isomers as is the absolute amount

of the nitrogen in each. Corn germ protein is able to maintain the biological value of 75.5 with considerably less nitrogen in the natural isomer of each essential amino acid than is the mixture of synthetics (assuming that the nitrogen of each unnatural isomer is totally wasted). The comparison is even more favorable to the natural protein on the basis of total absorbed nitrogen of the natural isomers; for the true digestibility of the protein is only 77%, while that of the amino acid mixture is 97% (table 3).

Putting the best face possible on the comparison of a synthetic protein with the natural one which it imitates in all its best qualities, the nutritional economy of the former makes a poor showing.

Beefsteak

This protein offers the advantage of much less alimentary waste. An attempt was made to effectuate the correction of the nitrogen of the unnatural isomers of a mixture imitating its protein, in the same way as was done successfully with the mixture imitating corn germ protein. The correction, however, was calculated and applied in one single period of 5 days — 3 days before adjustment and 2 afterward. Applied to the preliminary period the calculated increase of nitrogen from unnatural isomers necessary to reproduce the B.V. of the meat, on the assumption that all of it would be excreted, was 1.127 gm. The *dl* synthetics were increased accordingly, without changing the basic forms, and the resulting distribution of the natural isomers shown in table 6 was obtained. As may be seen the actual sum of the contributions from all the unnatural isomers fell slightly below the predicted value.

While the (relative) percentage change in the essentials is only 10 to 12% for the basics and 15 to 17% for the *dl* forms (cols. 3 and 5), the partial contribution which each makes to the absorbed nitrogen of all the natural isomers (cols. 4 and 7) is again considerably higher from the artificial than for the natural protein (about 70% for the basics and 30% for *dl* forms). Notwithstanding this, correction having

been made for all the unnatural nitrogen in the amino acid mixture, the former falls several points short of duplicating the biological value of the latter (table 7). If it had been possible to continue the experiment a few days longer, a second and more exact correction could have been made. The inevitable conclusion is that synthetic amino acids are nutritionally uneconomical.

TABLE 6

Distribution of nitrogen of natural isomers of essential amino acids in beefsteak and in the mixture of synthetic essentials (after adjustment).

	IN BEEFSTEAK PROTEIN			IN MIXTURE OF SYNTHETICS		
	Gm N/ 100 gm protein	Per cent of total	Gm each in av. absorbed	Gm N/ 100 gm mixture	Per cent of total	Gm each in av. absorbed
Arginine	3.25	32.7	0.709	3.82	36.2	1.206
Histidine	1.19	11.9	0.258	1.40	13.3	0.453
Isoleucine	0.59	5.9	0.129	0.52	4.9	0.164
Leucine	1.39	14.0	0.304	1.25	11.8	0.392
Lysine	1.54	15.5	0.336	1.80	17.1	0.570
Methionine	0.39	3.9	0.085	0.34	3.3	0.110
Phenylalanine	0.30	3.0	0.066	0.27	2.5	0.083
Threonine	0.60	6.0	0.131	0.54	5.1	0.165
Tryptophane	0.20	2.0	0.044	0.18	1.7	0.056
Valine	0.47	4.7	0.104	0.41	4.0	0.132
Totals	9.92	99.9	2.164	10.53	99.9	3.331
				Total N of unnatural isomers		1.102

The failure of the amino acid mixture in this experiment to duplicate the biological value of the protein, even under the most favorable conditions, might be explained by faulty analysis of the hydrolysate or by the fact that the nonessentials, being already formed in the protein and, possibly already in polypeptid combination with essentials to a greater extent than in corn germ, confer an advantage in time saved for their production as well as in conservation of essentials, or finally by the difficulty of computing the correction factor accurately in the middle of a period before all the analytical data were available. There is some reason to believe that the lysine de-

termination adopted was too low, and lysine, as shown in paper II (Murlin, Edwards, Hawley and Clark, '46b) is retained better than any other of the synthetic essentials when fed singly as an adjuvant to egg or soy bean protein. Indeed, as will be shown in a later publication (Hawley, Edwards, Clark and Murlin paper V, '46), this amino acid seems to induce considerable retention of other nitrogen than its own.

That the correction factor, equal to the total nitrogen of the unnatural isomers present in the mixture, was excreted as expected will be seen from the fractionation of the urines. The total nitrogen was partitioned by direct determination

TABLE 7

Biological value of beefsteak protein and of the amino acid mixture (after adjustment by increase of dl forms according to calculation below). Averages for 8 men.

	a	b	c	d	e	f	g	h	i	j	k	N-bal.
	Test protein fecal N	No-protein fecal N	(a–b) Fecal waste N	Test protein N eaten	(d–c) Absorbed N	True digestibility %	Test protein urine N	No-protein urine N	(g–h) Urinary waste N	$\frac{i \times 100}{e}$	$\frac{k}{B.V.}$	gm
Protein	1.057	1.028	0.029	3.889	3.860	99	2.378	2.040	0.338	8.7	91.3	+ 0.454
Amino acid Prelim.	1.039	1.028	0.011	3.946	3.935	99	3.411	2.040	1.371	34.8	65.2	— 0.502
Amino acids adjusted	1.039	1.028	0.011	4.667	4.656	99	3.689	2.040	1.526	32.8	67.2	— 0.061
Correction for all N of unnatural isomers:					1.102		1.102					
					3.554		2.587	2.040	0.547	15.4	84.6	— 0.061

Calculation for x in formula (1) p. 723:

$$91.3 = \frac{(3.935-x) - ((3.411-x)-2.040) \times 100}{(3.935-x)}$$

$$91.3(3.935-x) = (3.935-x) - ((3.411-x)-2.040) \times 100$$

$$-91.3x = (-3.411 + 2.040 + .342)100$$

$$91.3x = 102.9$$

$$x = 1.127$$

TABLE 8

Distribution of the nitrogen of the urine from beefsteak protein and the amino acid mixture imitating it before and after adjustment.

All quantities are averages for the squad of 8 men.

	TOTAL N	UREA N		AMMONIA N		AMINO ACID N		CREATININE N		URIC ACID N		MINED N UNDETER-	
		gm	%	*gm*	%	*gm*	%	*gm*	%	*gm*	%	*gm*	%
Beefsteak (Aug. 15)	2.545	1.152	45.3	0.355	13.9	0.232	9.1	0.527	20.7	0.148	5.8	0.132	5.2
Am. acid mixture (Aug. 17)	3.404	1.549	45.5	0.619	18.2	0.410[1]	12.1	0.510	15.0	0.096	2.8	0.219	6.4
Am. acid mixture after adjustment (Aug. 19 and 20)	3.689	1.476	40.0	0.832	22.6	0.550	15.0	0.522	14.2	0.110	3.0	0.181	5.0

[1] Approximately 43% of this nitrogen or 27% of the nitrogen of unnatural isomers fed was found by Dr. Bartlett to be of the *d* form.

of urea, ammonia, amino acids, creatinine and uric acid, and, by difference, of the undetermined portion, on one or two of the last days of the beefsteak period, the preliminary amino acid period and the final or adjusted amino acid period (table 8).[8] Noticeable differences between the beefsteak and amino acid periods are found in all columns except that for the absolute amounts of creatinine. The increases in urea and, still more, in the urea plus ammonia fraction, both absolute and relative, are significant. The sum of these two in the two amino acid periods is 63.7 and 62.6%, respectively, of the total nitrogen, while in the beefsteak period it is only 59.2%. Increasing ammonia reflects the greater acidity of the final mixture over the preliminary one and of the latter over that of the natural digestive products. The weight of amino acid nitrogen increases more rapidly from period to period than does the total nitrogen, as shown by the advancing percentages.

[8] Results were available for only a single day of the first two periods. For this reason a small discrepancy will be found between the total nitrogens of the urines of these periods in this table and in table 7.

This of course must mean that more undeaminated amino acids are passing through the kidney as these already digested products reach the blood stream in greater concentration.

Diminishing percentages of creatinine and uric acid nitrogen are expected because, whereas the beefsteak contains definite precursors of both products, creatine and nucleic acids, the amino acid mixture contained only arginine as a possible precursor of creatine. It was not sufficient apparently to overbalance the preformed creatine of the beefsteak.

But it is the sum of the first three fractions with which we are principally concerned, i.e., the sum of the deaminated and undeaminated amino nitrogens appearing in the urine. How well does the difference between these sums for the beef protein period and the final amino acid period agree with the correction factor representing the nitrogen of all the unnatural isomers of the *dl* forms in the final period? The sum for the former is 1.739 gm per man; for the latter it is 2.858 gm per man; and the difference is 1.119 gm. The correction in table 7 is 1.102. The nitrogen intake was increased in the final amino acid period over the beefsteak period by only 0.778 gm per man per day (table 7), but the nitrogen of the *dl* forms amounted to 2.204 gm, and that of the unnatural isomers in this 1.102 gm. It is unbelievable that the agreement between the sum of the nitrogens of the unnatural isomers in the total absorbed nitrogen and the sum of the extra nitrogens in the three fractions of the urine can be accidental. To the writers it means that the unnatural isomers are not available for retention when sufficient amounts of the natural ones are present.

SUMMARY AND CONCLUSIONS

In ten comparisons in human subjects of the proteins of whole egg, yeast, cottonseed meal, corn germ flour, beefsteak and haddock, with mixtures of the essential amino acids, compounded in the proportions one to another in which they occur in the proteins and supplying as much nitrogen as the pro-

tein, none of the mixtures possessed a biological value so high as that of the protein by from 10 to 40%.

The mixtures contained arginine, histidine and lysine in the natural *l* (+) form and as the monohydrochloride salt. All the other acids isoleucine, leucine, methionine, phenylalanine, threonine, tryptophane and valine were available in sufficient amounts only as the *dl* synthetic products.

In two experiments based on the hypothesis that if sufficient amounts of the natural isomers of the *dl* forms were supplied the unnatural isomers would be wasted, it was possible to duplicate closely the biological value of the proteins. A preliminary experiment was required to furnish data for calculation of the amount of nitrogen from unnatural isomers which would need to be excreted and subtracted from both sides of the balance sheet to produce the B.V. of the protein. Supplying these amounts in the *dl* isomers in a second experiment and deducting all the nitrogen of the unnatural isomers from both absorbed and urinary nitrogen of the amino acid period gave the predicted B.V. in one experiment and approached it in the other.[9] Correction in this manner for nitrogen representing no nutritional synthetic value has been practised regarding the purine compounds in beverages and would be in order in the case of medicinal agents or urea administered in the course of an experiment as a test substance for kidney function. The unnatural isomers in these experiments insofar as they escape deamination, belong to this class of dispensable compounds; while insofar as they are deaminated and can be recognized as contributing extra nitrogen to the urea and ammonia fractions of the urine, they are in the same class as nonessential amino acids.

The conclusion follows, that the use of *dl* synthetic amino acids, even those of the essential group, is nutritionally uneconomical.

[9] A third experiment (periods 6–iii and iv of table 1) gave the predicted B.V. precisely, but because it was based on earlier and less satisfactory analyses of the proteins concerned it is not reported in detail.

ACKNOWLEDGMENT

The authors wish to express their appreciation of the conscientious and intelligent cooperation of the following volunteers from the Civilian Public Service who participated as subjects, and many of them as technical assistants, in different parts of the work represented by the three papers of this series: Raymond Stanley, Frank Hastings, Oral Fisher, Arthur Dole, Peter Watson, Robert Dick, James McWhirter, Theodore Horvath, John Heil, Amos Culp, Hobart Mitchell, Harry Kadet, Berle Miller, William Lowden, Irvine Eller, George Spicer, Robert Long, Merle Whitaker, Ronald Ortmayer, Leo Baldwin and Albert Benglen.

LITERATURE CITED

ALBANESE, A. A. 1944 The utilization of *d* amino acids by man. I. Tryptophane, methionine and phenylalanine. Bull. Johns Hopkins Hosp., vol. 75, p. 175.

ALLISON, J. B., AND J. A. ANDERSON 1945 The relation between absorbed nitrogen, nitrogen balance and biological value of proteins in adult dogs. J. Nutrition, vol. 29, p. 413.

BAUER, C. D., AND C. P. BERG 1943 The amino acids required for growth in mice and the availability of their optical isomers. J. Nutrition, vol. 26, p. 51.

BERG, C. P. 1942 The metabolism of the *d* amino acids. Fed. Proc., vol. 1, p. 281.

DU VIGNEAUD, V., R. R. SEALOCK AND C. VAN ETTEN 1932 The availability of *d* tryptophane and its acetyl derivative to the animal body. J. Biol. Chem., vol. 98, p. 565.

HAWLEY, E. E., L. E. EDWARDS, L. C. CLARK AND J. R. MURLIN 1946 Biological value of proteins in relation to the essential amino acids which they contain. V. Effect of these amino acids, compared with additional egg protein as supplements to an egg diet, on fecal nitrogen and on biological value. In preparation.

HOLT, L. E., JR., AND A. A. ALBANESE 1945 Amino acid metabolism in health and disease. O.S.R.D. Progress Report no. 8, June 1, 1945.

MITCHELL, H. H. 1938 The biological value of the proteins and a criticism of the methods of its determination. Estratto Dagli Atti del VII Convego, Roma 26 Settembre — 2 Ottobre 1937-XV. Reale Accademia D'Italia, Roma.

MITCHELL, H. H., AND JESSIE R. BEADLES 1944 Corn germ: a valuable protein food. Science, vol. 99, p. 129.

MURLIN, J. R., L. E. EDWARDS, E. E. HAWLEY AND L. C. CLARK 1946a Biological value of proteins in relation to the essential amino acids which they contain. I. The endogenous nitrogen of man. J. Nutrition, vol. 31, p. 533.

———— 1946b Biological value of proteins in relation to the essential amino acids which they contain. II. Interconvertibility of biological values illustrated by supplementing egg and soy protein with essential amino acids. J. Nutrition, vol. 31, p. 555.

ROSE, W. C. 1938 The nutritive significance of the amino acids. Physiol. Rev., vol. 18, p. 109.

THOMAS, K. 1930 Biological values and the behavior of food and tissue proteins. J. Nutrition, vol. 2, p. 419.

TOTTER, J. R., AND C. P. BERG 1939 The influence of optical isomers on the utilization of tryptophane, histidine and lysine for growth in the mouse. J. Biol. Chem., vol. 127, p. 375.

VAN SLYKE, D. D. 1942 Physiology of the amino acids. Science, vol. 95, p. 259.

STUDIES ON THE COMPARATIVE NUTRITIVE VALUE OF FATS

VII. GROWTH RATE WITH RESTRICTED CALORIES AND ON INJECTION OF THE GROWTH HORMONE [1]

HARRY J. DEUEL, JR., CORNELIA HENDRICK, AND MARY E. CROCKETT

Department of Biochemistry and Nutrition, University of Southern California School of Medicine, Los Angeles

THREE FIGURES

(Received for publication January 12, 1946)

The rate of growth has usually been considered as an index of the nutritional value of a food. Thus, the food or foodstuff which promotes the more rapid growth has been considered to have the higher nutritive value. Schantz, Elvehjem and Hart ('40) originally reported that butter fat possesses a specific nutritive property not present in vegetable fats since greater growth is obtained in weanling rats over 6-week periods on diets of homogenized liquid skim milk and butter fat than when coconut, corn, cottonseed or soy bean oil was used in place of butter. The greater rate of growth observed by these investigators was always associated with a greater food consumption.

Deuel, Movitt, Hallman and Mattson ('44) found no differences in the growth rate of weanling rats fed a mixture of mineralized skimmed milk powder, vitamin supplements and

[1] This work was carried out under a research grant from The Best Foods, Inc. The authors wish to acknowledge the helpful advice of Prof. Anton J. Carlson of the University of Chicago, of Prof. Arthur W. Thomas of Columbia University and of Dr. H. W. Vahlteich of The Best Foods, Inc., during the course of the experiments.

fat, irrespective of whether butter or margarine fat, corn, cottonseed, olive, peanut or soy bean oil was the fat employed. In these experiments not only were the increases in body weight similar in the various dietary groups, but also bone growth was shown to be identical. No appreciable differences in food consumption were noted on the various diets. The specific effect of the saturated fatty acid fraction of butter on growth as postulated by Schantz, Elvehjem and Hart ('40) has not been confirmed (Henry, Kon, Hilditch and Meara, '45; Jack, Henderson, Reid and Lepkovsky, '45). Also the increased requirement of premature rats for butter fatty acids has been denied by Zialcita and Mitchell ('44) and by Deuel and Movitt ('45).

It is probable that flavor plays an important role in the amount of food consumed and hence indirectly on the extent of growth. Rats were found to prefer a butter-skimmed milk powder diet to one in which a bland vegetable fat was employed (Deuel and Movitt, '44). Furthermore, with two diets containing the same vegetable fat, the rat prefers one to which diacetyl or commercial butter flavor has been added to the extent of 1.2 parts per million to one which is unflavored. Although differences in food consumption and in growth were not noted in the earlier tests (Deuel et al., '44) for the groups receiving the butter diet or those containing the vegetable fats, it is probable that this flavor preference may account for the higher food consumption with the resultant superior growth that has in some cases been reported on the butter diets where ad libitum feeding was employed.

On artificial diets containing 48% of lactose, Boutwell, Geyer, Elvehjem and Hart ('44) reported a lower growth associated with a markedly reduced food intake when corn oil was the fat used as compared with tests where butter was employed. When diacetyl was added to the corn oil, food consumption was slightly increased and the difference between the butter and corn oil group decreased by 40%. Although admitting that off flavor or rancidity will have an adverse effect on food consumption, these authors state:

On purified diets the true explanation for the superior growth-promoting value of one substance over another must lie in the existence of specific compounds in that superior nutrient which results in a more favorable physiological response such that the animal grows at a faster rate and hence consumes more ration.

In the present tests the extent of growth has been augmented by the injection of growth hormone. If any essential component were lacking in sufficient amount in the vegetable fat diets to permit such augmented growth, such a deficiency should then become apparent. On the other hand, the response of rats to diets given in amounts insufficient for normal growth has also been followed in order to determine whether any differences in efficiency of the fat obtains under such conditions.

EXPERIMENTAL

Twenty-one-day weanling albino female rats of the Sprague Dawley strain were used. There were 10 rats in each group. In the experiments with restricted food intake, the rats were given a daily quota of 60% of the daily food consumption of control rats receiving the butter diet where ad libitum feeding was employed. The amount of food fed daily during each week, respectively, of the 9-week period of restricted food intake was as follows: 3.1, 4.4, 5.3, 5.6, 5.7, 6.2, 6.0, 6.2, and 6.2 gm. Food was given ad libitum from the tenth to twelfth weeks. The diets were approximately isocaloric and contained optimum quantities of the B vitamins, liver concentrate, and fat-soluble vitamins in addition to those in the skimmed milk powder. The composition of the diets is recorded in table 1.

The diets employed in the hormone tests were similar in composition without the addition of the added vitamins to the skimmed milk powder.

A growth hormone powder prepared from the anterior pituitary gland which assayed 1700 rat (growth) units per gram was used in these experiments.[2] A solution was prepared

[2] This was kindly furnished to us by Dr. Oliver Kamm, Parke, Davis and Co., Detroit, Michigan. Dr. Kamm stated that the figures for potency were based on the usual biological method for determination.

from the powder at frequent intervals by addition of an alkaline aqueous solution, adjustment of the pH to 8.5 and filtration of the undissolved material. Normal butyl alcohol

TABLE 1

The composition of diets used in studies where food intake was restricted.

COMPONENT		DIET 55b	DIET 55c
Mineralized skimmed milk powder [1]		70.3	66.2
Added minerals (per 100 lbs.)			
$MnSO_4 \cdot 7\ H_2O$	3.455 gm		
$CuSO_4 \cdot 5\ H_2O$	3.340 gm		
$Fe \cdot C_6H_8O_7 \cdot 3\ H_2O$	45.357 gm		
Added vitamins (per 100 lbs.) [2]			
Thiamine hydrochloride	1.27 gm		
Riboflavin	2.54 gm		
Pyridoxine	1.27 gm		
Calcium pantothenate	19.55 gm		
Choline chloride	77.40 gm		
Liver concentrate [3]	2390.00 gm		
Butter or margarine		29.7	
Added supplements per 1000 gm fat [4]			
Carotene	8.6 mg		
Vitamin A concentrate [5]	142.0 mg		
Viosterol	862.0 mg		
α-tocopherol	10.7 mg		
Commercial butter flavor	4.0 mg		
Vegetable oils (corn, cottonseed, peanut, soy bean, commercial hydrogenated fat) Supplements as above			25.6
Water			8.2
Calorie value (cal./100 gm)		480	476

[1] Challenge spray dried powder.

[2] This would give approximately the following in 10 gm of food; thiamine 200 μg; riboflavin, 400 μg; pyridoxine, 200 μg; calcium pantothenate 3 mg; and choline chloride 12 mg.

[3] Wilson 1 : 20 liver concentrate. This gives 0.37 gm per 10 gm food.

[4] Calculated to give the following amounts per 10 gm of food; carotene 25.5 μg, vitamin A, 84.5 I.U.; viosterol, 25.6 U.S.P. XI units; α-tocopherol, 31.8 μg.

[5] Vitamin A concentrate containing 200,000 I.U. per gm.

was used as a preservative in 1% concentration. Placebo solutions prepared in a similar manner without the growth hormone were used with the control groups. The solutions were injected intraperitoneally in 0.1 ml doses daily (6 days weekly).

Two series of tests were carried out at approximately 6-month intervals. In the first series, which consisted of five rats in each group (butter, cottonseed, margarine and soy bean diets without and with growth hormones), all animals survived and were in excellent condition throughout the test. In the second series a few of the rats in each group lost weight over several weeks after which some partially recovered. This occurred chiefly with the rats injected with hormone although in several instances it was found with the control rats. All rats of the litters so affected have been dropped from consideration. The results on corn oil were carried out only on the second series.

RESULTS

The average increases in weight of weanling rats receiving the various diets in restricted amounts during a 9-week period are presented in figures 1 and 2. Although weekly weighings were made, the averages are given only for the first, third, sixth and ninth week for the restricted period and for the tenth, eleventh and twelfth week during which ad libitum feeding was carried out. The data are for ten rats in each group.

The average body weight of the groups of rats at the start of the tests varied from 40.7 to 42.0 gm. At the end of 9 weeks of the restricted diets, the average total gain of the rats was as follows: butter (1) 92.5 ± 3.5 gm; corn oil (2) 95.5 ± 3.3; cottonseed oil (3) 103.3 ± 2.5; margarine (4) 90.7 ± 0.7; peanut oil (5) 97.8 ± 3.3; soy bean oil (6), 102.4 ± 2.1 and the commercial hydrogenated fat (7), 89.2 ± 2.4.

There was an immediate response to the ad libitum feeding with a marked increase in the growth rate. After the 3 weeks of ad libitum feeding, the total increase in weight had reached

the following figures: (1) 129.7, (2) 122.1, (3) 137.7, (4) 124.3, (5) 132.9, (6) 138.8 and (7) 124.5 gm.

The average gains in weight of rats receiving the injections of growth hormone daily and the control animals are shown in figure 3.

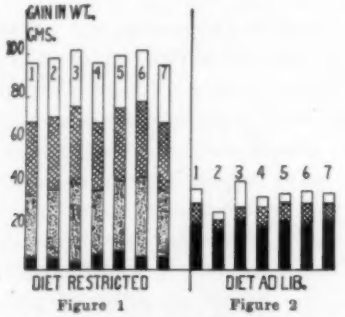


Figure 1 Figure 2

Fig. 1 The average gain in weight of female rats after 1 week (solid black), after 3 weeks (stippled), after 6 weeks (cross-hatched) and after 9 weeks (to top of blank space) on the diets containing the following fats and restricted in calories: 1, butter; 2, corn oil; 3, cottonseed oil; 4, margarine; 5, peanut oil; 6, soybean oil and 7, a commercial hydrogenated fat.

Fig. 2 The average total gain in weight of female rats recorded in figure 1 for tenth to twelfth weeks only where ad libitum feeding was employed. The gain for the first week is in solid black, after 2 weeks (cross-hatched) and after 3 weeks (to top of blank space).

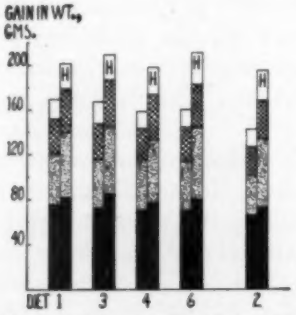


Fig. 3 The average gain in weight of female rats after 3 weeks (solid black), after 6 weeks (stippled), after 9 weeks (cross-hatched) and after 12 weeks (to top of blank space) on the following diets: 1, butter; 3, cottonseed oil; 4, margarine; 6, soy bean oil and 2, corn oil. The experiments on diet 2 were not all carried out simultaneously with the others. The columns marked "H" at the top are for the rats injected with growth hormone throughout and the other column was for the placebo-injected controls.

The average food intake and the relative efficiency of the food in the building of body tissue are summarized in table 2.

The relative efficiency in the utilization of the ingested food (gain in weight × 100: cal. consumed) is markedly higher in all cases where the growth hormone was administered. The increased efficiency (in per cent) in the groups injected with growth hormone as contrasted with the placebo-injected controls, was as follows: butter diet, 18.5; cottonseed oil diet, 23.8; margarine diet, 22.3; soy bean oil diet, 14.6; corn oil diet, 29.0.

TABLE 2

Summary table showing average gain in weight, average food consumption and ratio of increase in weight to calories consumed.

FAT IN DIET	NUMBER OF RATS	TOTAL GAIN IN WEIGHT	TOTAL FOOD CONSUMED	TOTAL CALORIES CONSUMED	EFFICIENCY [1]
		gm	*gm*		
Rats receiving placebo injection					
Butter	10	169.7	712.8	3420	4.97
Cottonseed	10	167.0	755.8	3590	4.65
Margarine	7	158.1	717.4	3410	4.63
Soybean	9	159.9	683.8	3250	4.92
Corn	9	142.1	668.2	3175	4.47
Rats receiving 10 units of growth hormone daily					
Butter	9	201.3	711.3	3415	5.89
Cottonseed	9	208.6	761.0	3625	5.76
Margarine	8	197.4	734.7	3490	5.66
Soybean	8	211.0	786.9	3740	5.64
Corn	9	194.4	710.3	3375	5.77

[1] $\frac{\text{Gm increase in weight}}{\text{Cal. consumed}} \times 100.$

DISCUSSION

No appreciable variation in the limited rate of growth of rats was found over a 9-week period irrespective of whether the skimmed milk diet contained a butter, a margarine, a commercial hydrogenated fat, or corn, cottonseed, peanut, or soy bean oil. Moreover, during a 3-week period of ad libitum feeding following the restricted period, the response of the rats by increased growth was immediate in all cases.

There is no indication from these tests that inclusion of butter in low calorie diets gives any superior response to that of vitamin-fortified vegetable oils, a margarine or a commercial hydrogenated fat.

In the experiments where growth hormone was injected, it was found that all groups were able to respond by an increased growth. The actual increase in growth in the corn oil tests (52.3 gm) was greatest in percentage although a similar response was obtained with the soy bean group (51.1 gm) while the increase in the butter group was only 31.6 gm. The somewhat lower gain in weight of the corn oil control group is probably to be ascribed to the fact that the experiments were carried out on only the second series of tests while with the other fats the tests were made with both series. The growth of the rats in all groups was lower in the second series of tests.

Although no appreciable variation was noted in the efficiency with which the different diets were utilized in the control tests, there was in all cases an increase in the efficiency of the utilization of the diets in the hormone-injected rats. The energy value of the injected hormone preparation itself was insignificant. If one assumes that 50% of the solids were present in the final solution as well as 1 mg of butyl alcohol in the daily quota, this would account for a maximum of 11 cal.; this quantity is inappreciable in comparison with the total caloric intake over the 84-day period which approximated 3500 cal. This increased efficiency in the hormone-treated rat is in line with the earlier results of Lee and Schaffer ('34) and of Marx et al. ('41–'42) who reported greater growth with an identical calorie intake.

It is our opinion that an excellent method to test the nutritive value of a diet is by its ability to support the additional growth occasioned by the injection of growth hormone. If the diet is inadequate, not only would one expect little additional growth on the injection of the hormone but also one might expect dietary failure sooner. Such a result has been reported by Ershoff and Deuel ('45) where a shorter length

of life obtained in rats deficient in vitamin A when hormone was injected than in the untreated controls. On the other hand, when vitamin A was also administered along with the basal diet, the growth was greater than in groups of rats on a similar intake of vitamin A without the growth hormone. The fact that a marked response to the growth hormone resulted in all groups in the present experiments would indicate that the diets were nutritionally satisfactory. This augmented dietary requirement can be as readily satisfied by vegetable fats as by butter.

SUMMARY

When rats were fed diets of mineralized skimmed milk powder and vitamin-fortified fats at a level of 60% of the ad libitum intake over a period of 9 weeks following weaning, the rate of growth was identical irrespective of whether the fat employed was a butter, a margarine, a commercial hydrogenated fat, or corn, cottonseed, peanut or soy bean oil. Moreover, there is no indication that differences exist in the ability of the rats to respond with increased growth during a 3-week period of ad libitum feeding following the period of restricted food intake in any dietary groups.

When growth hormone was injected, the augmented growth was as great or greater with the rats receiving the vegetable fat diets as with those receiving the butter diet. Since not only does increased growth not occur when growth hormone is injected in rats receiving deficient diets (i.e., vitamin A-free) but also the period of survival is decreased, the present results are interpreted as indicating that the various vegetable fats and margarine have an ability equal to butter in supporting such added growth requirements.

When growth hormone was injected, a greater efficiency in the utilization of the foodstuffs for growth was found.

LITERATURE CITED

BOUTWELL, R. K., R. P. GEYER, C. A. ELVEHJEM AND E. B. HART 1944 Effect of flavor on the nutritive value of fats. Proc. Soc. Exp. Biol. Med., vol. 55, p. 153.

DEUEL, H. J., JR., AND E. MOVITT 1944 Studies on the comparative nutritive value of fats. III. The effect of flavor on food preference. J. Nutrition, vol. 27, p. 339.

———— 1945 Studies on the comparative nutritive value of fats. V. The growth rate and efficiency of conversion of various diets to tissue in rats weaned at 14 days. J. Nutrition, vol. 29, p. 237.

DEUEL, H. J., JR., E. MOVITT, L. F. HALLMAN AND F. H. MATTSON 1944 Studies on the comparative nutritive value of fats. I. Growth rate and efficiency of conversion of various diets to tissue. J. Nutrition, vol. 27, p. 107.

ERSHOFF, B. H., AND H. J. DEUEL, JR. 1945 The effect of growth hormone on the vitamin A-deficient rat. Endocrinology, vol. 36, p. 280.

HENRY, K. M., S. K. KON, T. P. HILDITCH AND M. L. MEARA 1945 Comparison of the growth-promoting value for rats of butter fat, of margarine fat and of vegetable oils. J. Dairy Res., vol. 14, p. 45.

JACK, E. L., J. L. HENDERSON, D. F. REID AND S. LEPKOVSKY 1945 Nutritional studies on milk fat. II. The growth of young rats fed glyceride fractions separated from milk fat. J. Nutrition, vol. 30, p. 175.

LEE, M. O., AND N. K. SCHAFFER 1934 Anterior pituitary growth hormone and the composition of growth. J. Nutrition, vol. 7, p. 337.

MARX, W., M. E. SIMPSON, W. O. REINHARDT AND H. M. EVANS 1941–1942 Response to growth hormone of hypophysectomized rats when restricted to food intake of controls. Am. J. Physiol., vol. 135, p. 614.

SCHANTZ, E. J., C. A. ELVEHJEM AND E. B. HART 1940 The comparative nutritive value of butter fat and certain vegetable oils. J. Dairy Sci., vol. 23, p. 181.

ZIALCITA, L. P., JR., AND H. H. MITCHELL 1944 Corn oil and butter fat essentially equal in growth-promoting value. Science, vol. 100, p. 60.

STUDIES ON THE COMPARATIVE NUTRITIVE VALUE OF FATS

VIII. THE FAILURE OF ETHER EXTRACTION TO LOWER THE NUTRITIVE VALUE OF SKIMMED MILK POWDER IN DIETS CONTAINING VARIOUS VEGETABLE FATS [1]

HARRY J. DEUEL, JR., CORNELIA HENDRICK, ELI MOVITT, MARY E. CROCKETT, IRENE M. SMYTH AND RICHARD J. WINZLER

Department of Biochemistry and Nutrition, University of Southern California, School of Medicine, Los Angeles

ONE FIGURE

(Received for publication January 14, 1946)

In the first report of the present series of papers (Deuel, Movitt, Hallman and Mattson, '44) it was shown that no differences occurred in the rate of growth of male or female rats fed on diets of unextracted mineralized skimmed milk powder and vitamin-fortified fats irrespective of whether a butter, a margarine or corn, cottonseed, olive, peanut or soybean oil was the fat employed. In the original paper of Schantz et al. ('40), where the most decisive differences between the butter and vegetable fat groups were reported, unextracted liquid skimmed milk was used. In their later tests (Boutwell et al., '43) skimmed milk powder was used which had been extracted with diethyl ether for four 8-hour periods during which it was subjected to constant agitation.

In an editorial discussion on the nutritive value of butter fat (Anonymous, '44), a possible explanation was offered

[1] This work was carried out under a research grant from The Best Foods, Inc. The authors wish to acknowledge the helpful advice of Prof. Anton J. Carlson of the University of Chicago, of Prof. Arthur W. Thomas of Columbia University and of Dr. H. W. Vahlteich of The Best Foods, Inc., during the course of the experiments.

for the divergent results of the Wisconsin group and those of Deuel et al. ('44), namely, that the vegetable oil diets in the latter tests actually contained some butter fat since the skimmed milk powder used had 1.1% of residual fat. Boutwell et al. ('45) have also recently made a similar implication when they state that " . . . Deuel et al. (3, 4, 5) fed weanling rats an unextracted skimmilk powder ration . . ."

The amount of residual fat available to the rat from the unextracted skimmed milk powder seems far too small to be of consequence. Based on an average daily food intake of 10 gm, the total butter fat from the skimmed milk powder containing 1.1% lipid would amount only to 77 mg. This would give a maximum of 28 mg of saturated fatty acids of the fraction (C_{16}–C_{20} (Jack et al., '45)), which consists of palmitic and stearic acid. This is the fraction of butter which is considered by Schantz, Elvehjem and Hart ('40) to contain the fatty acids having the specific nutritive value. It is difficult to see how in addition to palmitic and stearic acids, there could be any appreciable quantity of hitherto unidentified fatty acids in sufficient amount to result in any improvement in the nutritive value of the diet. The optimum quantity of methyl linolate required for growth is probably about 100 mg daily.[2] It should also be mentioned that Jack et al. ('45) and Henry et al. ('45) have failed to demonstrate any specific nutritive value in this saturated fatty acid fraction of high molecular weight or in any other fraction of butter fat.

The present tests were designed to determine how completely fat could be removed from skimmed milk powder by the treatment employed by Boutwell et al. ('43) and also to ascertain whether under the experimental conditions which we have employed any differences would be observed in the growth of weanling rats fed such extracted skimmed milk powder combined with a butter, or a margarine or the vegetable fats instead of the unextracted product combined with these fats.

[2] Personal communication from Dr. G. O. Burr.

METHODS AND RESULTS

Experiments on extraction of fat from skimmed milk powder

In order to determine the effectiveness of diethyl ether in removal of the fat, approximately 700 gm of skimmed milk powder [3] was suspended in 3,000 ml of redistilled U.S.P. diethyl ether and vigorously agitated for 8 hours with a large paddle operated by an air stirrer so that the solid was kept in suspension. The ether was filtered off, the skimmed milk powder suspended in fresh ether, and the procedure repeated eight times. Samples of the skimmed milk powder were analyzed after each extraction by the Roese-Gottleib procedure. In one series the fat removed in the ether was also determined in each case. Extraction was also carried out with diethyl ether in a special Soxhlet apparatus [4] for three successive 24-hour periods followed by two 24-hour extractions with 95% ethyl alcohol. A combined diethyl ether-ethyl alcohol extraction over 48 hours was also used. The results are summarized in table 1.

In the tests recorded in table 1, only 12, 26 and 31% of the fat were removed by four successive 8-hour extractions with diethyl ether. Even after eight extractions of the skimmed milk powder, only 21, 38, and 48% were removed in different tests. This value almost exactly coincides with that found by 72 hours of continuous extraction of the skimmed milk powder on the Soxhlet apparatus using diethyl ether. A further extraction with warm ethyl alcohol also in the Soxhlet apparatus reduced the residual fat to as low as 0.35%. The most effective and quickest removal of fat would seem to be by the combined simultaneous extraction on the Soxhlet with alcohol and ether. After 48 hours extraction on the Soxhlet apparatus, the fat was reduced to 0.29%.

The ineffectiveness of diethyl ether as an extraction agent is further indicated by the results obtained on different

[3] Challenge spray dried skimmed milk powder was used.

[4] A specially built Soxhlet having an extraction chamber of 4-liter capacity built by Mr. E. Greiner was used.

batches of extracted skimmed milk powder used in the feeding tests. The residual fat content was found to be 0.84, 0.77, 0.70 and 0.61% in four different lots compared with a value of 0.87% in the unextracted sample.

TABLE 1

The effectiveness of continuous stirring with diethyl ether and of Soxhlet extraction with ether, alcohol or an alcohol-ether mixture in removing lipid from skimmed milk powder.

AIR STIRRER (DIETHYL ETHER)					SOXHLET				
Time of extraction	Fat content				Time of extraction	Solvent	Residual fat content		
	Sample 1 Residual	Sample 2 Residual	Sample 3				Sample 1	Sample 2	Sample 3
			Residual	Extracted					
hours	%	%	%	%	*hours*		%	%	%
0	1.01	1.01	1.00						
8	0.94	0.91	0.93	0.046	24	Ether	0.90	0.96	
16	0.86	0.93	...	0.039	48	Ether	0.91	0.81	
24	0.83	0.78	0.92	0.032	72	Ether	0.84	0.78	
32	0.75	0.70	0.88	0.027					
40	0.74	0.71	0.84	0.018	24	Alcohol [1]	0.45	0.54	
48	0.73	0.66	0.82	0.020	48	Alcohol	0.35	0.56	
56	0.67	0.60	0.80	0.015	24	Alcohol-ether [2]	...	...	0.34
64	0.63	0.53	0.79	0.015	48	Alcohol-ether	...	...	0.29
Total				0.212					

[1] Ether extraction for 72 hours followed by alcohol extraction.

[2] Alcohol-ether mixture used of such proportions so that ether would fill approximately half of the extracting chamber. The alcohol would then be distilled over to fill the chamber to the point of siphoning giving a 1:1 mixture of the solvents at that time.

Feeding experiments with diets made up of extracted skimmed milk powder and different fats

Feeding experiments were made with weanling male and female rats on diets of extracted skimmed milk powder to which was added a butter, a margarine, or corn, cottonseed, peanut or soybean oil. The diets and other experimental

procedures were the same as those in our original experiments (Deuel et al., '40) except that rats of the Sprague Dawley strain were used. Ten rats were used in each group. The gains in weight at 3, 6, 9, and 12 weeks are given in figure 1 and a summary of the data on efficiencies of the various diets is reported in table 2.

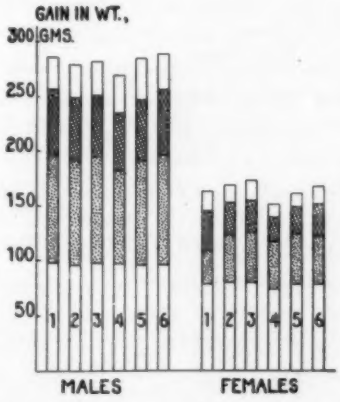


Fig. 1 The average gain in weight of male and female rats for 3 weeks (lower blank space), 6 weeks(stippled), 9 weeks (cross-hatched) and 12 weeks (upper blank space) is given for the following diets: 1, butter; 2, corn oil; 3, cottonseed oil; 4, margarine; 5, peanut oil; 6, soybean oil.

No differences in the rate of growth or in the total gain were noted in the diets with extracted skimmed milk powder irrespective of whether the fat used was a butter, a margarine or corn, cottonseed, peanut or soybean oil. The efficiency of utilization of the foodstuffs is the same within experimental error in all cases.

DISCUSSION

The divergent results obtained by Deuel et al. ('44) and Boutwell et al. ('43) cannot be ascribed to the use of unextracted skim milk powder by the former investigators. The extraction procedure on the skimmed milk powder employed by the latter group (diethyl ether for four successive

8-hour periods) removes only a small fraction of the total residual lipids in the milk powder. In addition, using a similar strain of rats employed by the Wisconsin group, it was shown

TABLE 2

The food consumption and efficiency of utilization of diets of mineralized-extracted skimmed milk powder with different fats when fed to rats over a 12-week period.

DIET	BODY WEIGHT		TOTAL GAIN [1]	TOTAL FOOD	TOTAL CALORIES	EFFICIENCY [2]
	Start	End				
	gm	*gm*	*gm*	*gm*		
Male rats						
Butter	42.5	328.1	285 6 ± 5.5	968.9	4545	6.28
Corn oil	42.6	322.2 (9)	278.9 ± 5.4	987.6	4635	6.02
Cottonseed oil	42.1	323.6	281.5 ± 6 6	972.2	4560	6.10
Margarine	42.4	311.1	268.7 ± 3.4	929.1	4357	6.17
Peanut oil	42.1	325.7 (9)	284.3 ± 5.9	979.2	4600	6.19
Soybean oil	42.1	331.7	289.4 ± 5.9	999.4	4688	6.18
Female rats						
Butter	42.8	206.2	163 4 ± 6.7	732.7	3436	4.75
Corn oil	43.3	211.8	168.5 ± 4.8	768 1	3602	4.68
Cottonseed oil	42.6	215.3	172.7 ± 5.8	795.2	3729	4.62
Margarine	43.9	195 0	151.1 ± 7.5	744.0	3489	4.33
Peanut oil	43.3	204.2	160.9 ± 3.9	788.5	3698	4.34
Soybean oil	42.9	210.3	167.4 ± 4.6	807.0	3788	4.42

[1] Including the standard error of the mean calculated as follows:

$$\sqrt{\frac{\Sigma d^2}{n}} \Big/ \sqrt{n}$$

where "d" is the deviation from the mean and "n" is the number of observations.

[2] Ratio of $\frac{\text{gm gain} \times 100}{\text{cal. consumed}}$.

that no differences in the rate of growth occur between butter and the vegetable fats when extracted skimmilk powder is used.

SUMMARY

Residual fat is removed from skimmed milk powder only to the extent of 12 to 31% by four successive 8-hour extractions with diethyl ether and to 21 to 48% when extraction is continued for four additional 8-hour periods when the mixture is constantly agitated. Continuous extraction for 72 hours with diethyl ether on a Soxhlet apparatus removes about 20% of the residual lipids while extraction with ethyl alcohol resulted in a lowering of 35% of the original content. The most effective extraction was made by simultaneous extraction with alcohol and ether.

No differences in rate of growth or in the total ultimate gain in weight over a 12-week period was observed when weanling male or female rats were fed extracted skimmed milk powder mixed with fat, irrespective of whether a butter or a margarine or corn, cottonseed, peanut or soybean oil was the fat used.

LITERATURE CITED

ANONYMOUS 1944 Nutritive value of butterfat. Nutrition Reviews, vol. 2, p. 267.

BOUTWELL, R. K., R. P. GEYER, C. A. ELVEHJEM AND E. B. HART 1943 Further studies on the growth-promoting value of butter fat. J. Dairy Sci., vol. 26, p. 429.

DEUEL, H. J., JR., E. MOVITT, L. F. HALLMAN AND F. MATTSON 1944 Studies on the comparative nutritive value of fats. I. Growth rate and efficiency of conversion of various diets to tissue. J. Nutrition, vol. 27, p. 107.

HENRY, K. M., S. K. KON, T. P. HILDITCH AND M. L. MEARA 1945 Comparison of the growth-promoting value for rats of butter fat, of margarine fat and of vegetable oils. J. Dairy Res., vol. 14, p. 45.

JACK, E. L., J. L. HENDERSON, D. F. REID AND S. LEPKOVSKY 1945 Nutritional studies on milk fat. II. The growth of young rats fed glyceride fractions separated from milk fat. J. Nutrition, vol. 30, p. 175.

SCHANTZ, E. J., C. A. ELVEHJEM AND E. B. HART 1940 The comparative nutritive value of butterfat and certain vegetable oils. J. Dairy Sci., vol. 23, p. 181.

THIAMINE, RIBOFLAVIN, NICOTINIC ACID, PANTOTHENIC ACID AND ASCORBIC ACID CONTENT OF RESTAURANT FOODS [1]

HERBERT P. SARETT,[2] MARGARET JEAN BENNETT, THOMAS R. RIGGS AND VERNON H. CHELDELIN

Department of Chemistry, Oregon State College, Corvallis, Oregon

(Received for publication December 17, 1945)

The vitamin content of the human dietary has been the subject of considerable study and speculation within recent years. Indirect information is obtainable from the analysis of vitamins in uncooked foods (Waisman and Elvehjem, '41; Cheldelin and Williams, '42). More direct studies have reported cooking losses and vitamin contents in foods prepared under controlled conditions which might be duplicated in household practice (Lane, Johnson and Williams, '42; Cheldelin and Williams, '43; McIntire, Schweigert and Elvehjem, '43; Oser, Melnick and Oser, '43).

Similar investigations of foods eaten in restaurants and in various institutions have emphasized the losses incurred during preparation and service of foods (Eakin and Gerrard, '43; Heller, McCay and Lyon, '43; Nagel and Harris, '43; Peterson, '44; Wertz and Weir, '44; Koch et al., '45). Less information has been published, however, regarding the amounts of B vitamins which are actually available to the

[1] This study was supported by the Nutrition Foundation, Inc., and by the Committee on Food Composition of the National Research Council.

Published with the approval of the Monographs Publication Committee, Oregon State College, Research paper no. 100, School of Science, Department of Chemistry.

[2] Present address: Tulane University School of Medicine, New Orleans, Louisiana.

restaurant-going public. The present work was undertaken for this purpose, as well as to compare the vitamin contents of foods from different grades of restaurants. It includes analyses for the thiamine, riboflavin, nicotinic acid, pantothenic acid and ascorbic acid contents of foods obtained from three Corvallis establishments representing three price groups.

This study is not extensive. Only one sample of each food was analyzed, and no figures on the variation among samples are available. The data, however, give no evidence of important differences in the vitamin contents of foods from the three restaurants, and show that adequate vitamin intakes may be obtained by judicious selection of meals.

EXPERIMENTAL

The foods for analysis were obtained from each restaurant at mealtime, so as to secure samples as served. They included foods comprising the regular meals as well as several popular a la carte items such as sandwiches, chili, etc. Individual servings of each item were immediately brought to the laboratory, where they were weighed and homogenized with several volumes of water in a Waring blendor to obtain representative samples.

Thiamine and pantothenic acid assays were performed on enzymatically digested samples, using Lactobacillus fermenti for thiamine (Sarett and Cheldelin, '44) and Lactobacillus arabinosus for pantothenic acid (Hoag, Sarett and Cheldelin, '45). Samples for riboflavin analyses were autoclaved with 0.1 N HCl for 15 minutes (Strong and Carpenter, '42) and assayed by the method of Snell and Strong ('39), using additional amounts of glucose and acetate in the medium as suggested by Stokes and Martin ('43). For nicotinic acid analyses, aliquots were autoclaved for 30 minutes in 1 N H_2SO_4, and assayed by the microbiological method of Sarett, Pedersen and Cheldelin ('45). The foods analyzed for ascorbic acid were blended with seven volumes of 1% metaphosphoric acid and assayed by the 2,6-dichlorophenolindophenol method of Loeffler and Ponting ('42). In the first

groups of foods studied, ascorbic acid analyses were carried out on most of the samples collected. Since most of the cooked foods contained less than 10 μg per gm, subsequent analyses were performed only on fruits, juices and some of the vegetables.

RESULTS

Values for the thiamine, riboflavin, nicotinic acid, pantothenic acid, and ascorbic acid content of the foods from the three restaurants are presented in tables 1, 2, and 3 in terms of micrograms or milligrams per individual portion. Restaurant A (table 1) is a hotel coffee shop, which represents a "better class" establishment in a small town; B (table 2) is a moderate restaurant which caters to campus personnel, and C (table 3) serves food lower in price and of seemingly poorer quality. The sampling of all three restaurants was performed during 1944 and 1945, when rationing may have had some influence upon the quality of foodstuffs obtainable. No marked differences were found among the vitamin contents of the foods served at the three restaurants.

Since each of these menus represents the food served at one particular meal and affords many different meal combinations, only approximate estimates may be made of an individual's vitamin intake at each restaurant. Also, food consumption at breakfast varies greatly, and individual likes and dislikes for certain food items (particularly bread) may influence the values obtained. At restaurant A the approximate ranges of intake of thiamine, riboflavin, nicotinic acid, and pantothenic acid for the various daily combinations of meals possible are 0.6–1.8, 1.4–2.3, 8–26 and 3.6–8.3 mg, respectively. For restaurant B these figures are 0.9–1.8, 1.2–2.1, 10–25, and 4.2–10.3 mg, respectively. At C the comparable amounts of the vitamins are 0.7–2.5, 0.7–2.7, 8–30, and 3.1–18 mg, respectively.

The thiamine intake obtainable from a varied diet at these restaurants tends to be lower than the value of about 1.5 mg per day, recommended by the Food and Nutrition Board of the National Research Council ('45), unless some of the thia-

TABLE 1

The vitamin contents of foods served in restaurant A.

FOODS	WEIGHT	THIAMINE	NICOTINIC ACID	RIBOFLAVIN	PANTOTHENIC ACID	ASCORBIC ACID
	gm	μg	μg	μg	μg	mg
Breakfast						
Tomato juice, canned	175	60	1800	37	350	35
Orange juice, fresh	170	153	660	41	257	102
Figs, canned	108	11	200	15	75	
Applesauce, canned	113	34	40	12	113	
Grapefruit, canned	157	80	310	17	110	30
Prunes	140	14	64	41	195	3
Cream of wheat, with cream	355		438	114	597	
Oatmeal with cream	360	338				
Pep	32	190	1150	35	150	
Post Toasties	31	68	325	16	62	
Cream for cereal	120	24	108	90	385	
Fried egg (1)	47	36	45	165	850	
Scrambled egg (1)	52	40	36	160	1190	
Boiled egg (1)	47	36	35	150	940	
Sausage	70	450	3400	260	980	
Ham	41	310	1900	125	450	
Bacon	20	52	900	60	335	
Waffle (1)	71	100	960	185	385	
Hot cakes (3)	219	240	2300	370	100	
Toast, 2 slices	46	22	460	28	130	
Doughnuts (2)	81	40	630	40	315	
Lunch						
Macaroni and cheese	185	74	610	315	407	
Sirloin tips, with vegetables	230	69	4840	345	505	10
Hamburger	103	41	4950	206	392	
Salmon, canned	112	5	6280	168	426	
Short ribs of beef, boiled, (183 gm with bones)	90	15	2880	144	135	
Potatoes, mashed	84	42	680	45	296	0
Mixed vegetables — turnip, onion, carrot	182	36	1055	76	310	3.6
String beans	75	23	270	56	53	0
Potato salad	111	33	880	58	520	0
Gelatin salad	60	10	90	23	42	0
Chili, bowl	260	143	2860	197	364	7
Bread, 2 slices	52	25	400	28	140	
Milk, individual bottle	240	132	168	500	1000	
Dinner						
Rice tomato soup	175	10	470	26	88	1.5
Peach and cottage cheese salad	83	10	250	100	100	1
Ham, baked with pineapple	81	610	2200	137	420	
Chicken pot pie	156	94	4370	156	470	
Veal loaf, baked	146	146	4100	340	890	
Halibut, grilled	120	110	9100	100	390	
Link sausage	93	510	2320	150	450	
Potato, boiled	101	60	570	22	300	7
Corn, whole kernel	71	16	500	37	210	3
Shrimp Newberg	176	79	780	210	769	
Crab Louie	275	165	1200	220	1800	
Rolls (2)	67	124	1540	167	210	
Peach pie	187	205	2170	93	225	
Apple pie	138	180	1300	77	165	
Cake, white	73	22	170	62	220	

TABLE 2

The vitamin contents of foods served in restaurant B.

FOODS	WEIGHT	THIAMINE	NICOTINIC ACID	RIBOFLAVIN	PANTOTHENIC ACID	ASCORBIC ACID
	gm	μg	μg	μg	μg	mg
Breakfast						
Tomato juice, canned	188	150	1220	28	325	47
Orange juice, fresh	193	280	830	33	465	110
Grapefruit juice, canned	181	132	235	16	200	48
Prunes	119	6	715	20	200	0
Cereal, Ralston type and cream	275	146	2100	173	600	
Corn flakes	33	220	600	15	55	
Bran flakes, 40%	33	220	3100	51	290	
Fried egg (1)	48	57	36	70	1420	
Poached egg (1)	54	75	....	79	1170	
Bacon	15	114	1100	30	225	
Waffle (1)	105	160	1450	140	800	
Hot cakes (3)	215	290	2800	175	1270	
Toast, 2 slices	50	133	410	23	152	
Lunch						
Celery soup	200	42	200	230	920	
Meat pie	207	75	6300	310	750	
Potatoes, mashed, and gravy	101	73	1100	70	350	0
Green peas	85	40	700	48	102	
Spanish corn	147	57	1250	75	300	
Spinach	119	25	580	130	70	
Turnips, creamed	101	27	300	30	170	
Salad, carrot and cabbage	110	10	210	27	310	8
Apple pie	129	85	890	65	130	
Custard pie	138	27	480	193	1300	
Hamburger sandwich	140	103	3980	137	500	
Milk, individual bottle	240	86	168	370	960	
Dinner						
Cream of potato soup	210	105	900	245	710	
Swiss steak	95	65	4750	145	350	
Dinner steak, rare	116	130	6000	185	830	
Cauliflower	57	49	305	74	350	
String beans	70	28	155	30	45	
Potatoes, mashed, and gravy	98	83	1420	45	340	
Cottage cheese salad	96	35	71	182	200	
Rhubarb pie	152	213	1530	110	190	
Cake, chocolate	67	14	215	35	135	
Ham sandwich, cold	126	315	2950	230	655	

mine rich foods are consumed regularly. Thiamine is readily lost in cooking (Lane, Johnson and Williams, '42; Nagel and Harris, '43) and the interval between cooking and service may also contribute to further losses (Nagel and Harris, '43).

The riboflavin level in these diets is influenced considerably by the amount of milk consumed. The inclusion of one glass of milk per day with an average diet at these establishments

TABLE 3

The vitamin contents of foods served in restaurant C.

FOODS	WEIGHT	THIAMINE	NICOTINIC ACID	RIBOFLAVIN	PANTOTHENIC ACID	ASCORBIC ACID
	gm	*μg*	*μg*	*μg*	*μg*	*mg*
Breakfast						
Tomato juice, canned	167	70	1500	43	234	25
Orange juice, canned	195	154	515	53	350	58
Oatmeal and cream	263	258	445	180	640	
Fried egg (1)	42	63	61	128	1565	
Ham	55	300	5100	204	990	
Bacon	31	195	1750	58	255	
French toast	125	183	1330	332	1100	
Doughnuts	78	90	515	94	300	
Lunch and dinner						
Vegetable soup	132	30	570	25	185	11
Meat loaf	244	180	2500	178	810	
Hamburger	126	176	6500	272	960	
Wieners and sauerkraut	151	74	1620	92	440	
Red snapper	129	135	3640	166	465	
Pork steak	141	815	2900	271	830	
Salmon	103	120	6700	128	710	
Sausage	83	480	2020	155	550	
Oysters, fried	152	595	3800	284	1450	
Liver	113	400	12400	455	8000	
Potatoes and gravy	99	68	740	52	297	6
Turnips	89	62	545	23	115	0
Vegetable salad	70	58	142	34	112	5
Bread, 2 slices	49	115	1360	90	202	
Pumpkin pie	106	61	335	188	550	
Berry pie	137	42	490	41	252	
Hot beef sandwich	193	340	5150		870	
Milk, individual bottle	237	111	152	520	1070	

allows sufficient daily riboflavin intake to satisfy the National Research Council's recommendations of 1.6–2.0 mg ('45).

The nicotinic acid content of these diets, in most combinations, also meets the allowance of 12–15 mg per day recommended by the National Research Council ('45). In addition to the nicotinic acid figures shown above, as much as 1 mg per cup of coffee (which was not included in the present analyses) may be added to the intake (Teply, Krehl and Elvehjem, '45) if it is assumed that the materials in coffee which stimulate the growth of L. arabinosus possess antipellagric value.

Since the human requirements for pantothenic acid are not known, the values given for this vitamin cannot be interpreted at present.

The recommended daily intake of 75 mg of ascorbic acid (National Research Council, '45) can only be met in all three restaurants by frequent consumption of orange juice. The only respect in which restaurant C appears to be inferior to the others is in its use of canned orange juice, which is lower in ascorbic acid than fresh orange juice. The extreme lability of ascorbic acid makes restaurant salads and cooked vegetables a poor source of this vitamin. This is particularly evident in the variable values of 0 to 7 mg for different portions of potatoes.

SUMMARY

Single portions of food served for each meal at three restaurants were analyzed for thiamine, riboflavin, nicotinic acid, pantothenic acid, and ascorbic acid. Although the food served by the three restaurants varied with respect to cost and attractiveness as served, there were no marked differences in the vitamin content.

From estimates of daily food intake, it was calculated that riboflavin and nicotinic acid requirements were readily met by varied diets in all three restaurants. Thiamine intake on these diets were equal to the recommended daily allowances, if care was taken in the selection of foods. Judging from the data obtained ascorbic acid requirements could be maintained only by the inclusion of fresh fruit juices.

The vitamins most readily lost in cooking and handling of restaurant foods were thiamine and ascorbic acid.

LITERATURE CITED

CHELDELIN, V. H., AND R. J. WILLIAMS 1942 The B vitamin content of foods. The University of Texas Publication, no. 4237, p. 105.

CHELDELIN, V. H., AND R. R. WILLIAMS 1943 Studies of the average American diet. II. Riboflavin, nicotinic and pantothenic acid content. J. Nutrition, vol. 26, p. 417.

EAKIN, R. E., AND E. GERRARD 1943 Vitamin content of naval flight rations as affected by the method of cooking. Naval Med. Research Institute, Report no. 1, 1943.

HELLER, C. A., C. M. MCCAY AND C. B. LYON 1943 Losses of vitamins in large-scale cookery. J. Nutrition, vol. 26, p. 377.

HOAG, E. H., H. P. SARETT AND V. H. CHELDELIN 1945 The use of Lactobacillus arabinosus 17 for assay of pantothenic acid. Ind. Eng. Chem., Anal. Ed., vol. 17, p. 60.

KOCH, F. G., F. STREIGHTOFF, H. E. MUNSELL, B-A. BEN DOR, M. L. ORR, R. CAILLEAU, M. H. LEONARD, S. R. EZEKILL AND R. KORNBLUM 1945 The effect of large-scale methods of preparation on the vitamin content of food. I. The ascorbic acid, niacin, thiamin, and riboflavin content of potatoes. Nutrition Laboratory, Office of the Pentagon Post Restaurant ("Officer"), Publication no. 1.

LANE, R. L., E. JOHNSON AND R. R. WILLIAMS 1942 Studies of the average American diet. I. Thiamine content. J. Nutrition, vol. 23, p. 613.

LOEFFLER, H. J., AND J. D. PONTING 1942 Ascorbic acid determination in fruits and vegetables, fresh, frozen, or dehydrated. Ind. Eng. Chem., Anal. Ed., vol. 14, p. 846.

MCINTIRE, J. M., B. S. SCHWEIGERT AND C. A. ELVEHJEM 1943 The retention of vitamins in veal and lamb during cooking. J. Nutrition, vol. 26, p. 621.

NAGEL, A. H., AND R. S. HARRIS 1943 Effect of restaurant cooking and service on vitamin content of foods. J. Amer. Dietet. Assoc., vol. 19, p. 23.

OSER, B. L., D. MELNICK AND M. OSER 1943 Influences of cooking procedure on retention of vitamins and minerals in vegetables. Food Research, vol. 8, p. 115.

PETERSON, W. J. 1944 Cooking losses at army and navy training camps at land grant institutions. National Cooperative Project, Southern Region Progress Note no. 10. (Issued by N. C. Agric. Exp. Sta.)

Recommended dietary allowances, revised, 1945 National Research Council Reprint and Circular Series no. 122.

SARETT, H. P., AND V. H. CHELDELIN 1944 The use of Lactobacillus fermenti 36 for thiamine assay. J. Biol. Chem., vol. 155, p. 153.

SARETT, H. P., R. L. PEDERSEN AND V. H. CHELDELIN 1945 Microbiological assay methods for nicotinic acid. Arch. Biochem., vol. 7, p. 77.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. Ind. Eng. Chem., Anal. Ed., vol. 11, p. 346.

STOKES, J. L., AND B. B. MARTIN 1943 Influence of buffer and glucose in the Lactobacillus casei assay for pantothenic acid. J. Biol. Chem., vol. 147, p. 483.

STRONG, F. M., AND L. E. CARPENTER 1942 Preparation of samples for microbiological determination of riboflavin. Ind. Eng. Chem., Anal. Ed., vol. 14, p. 909.

TEPLY, L. J., W. A. KREHL AND C. A. ELVEHJEM 1945 Studies on the nicotinic acid content of coffee. Arch. Biochem., vol. 6, p. 139.

WAISMAN, H. A., AND C. A. ELVEHJEM 1941 The vitamin content of meat. Burgess, Minneapolis.

WERTZ, A. W., AND C. E. WEIR 1944 The effect of institutional cooking methods on the vitamin content of food. J. Nutrition, vol. 28, p. 255.

THE COMPARATIVE VALUE OF CERTAIN DIETARY PROTEINS FOR HEMOPOIESIS IN THE RAT[1]

JAMES M. ORTEN AND ALINE UNDERHILL ORTEN
Department of Physiological Chemistry, Wayne University College of Medicine, Detroit

(Received for publication January 24, 1946)

There is adequate evidence demonstrating that, although hemoglobin formation has a "high priority call" on available protein in the organism, the feeding to the rat of a diet containing either an inadequate amount of dietary protein (Orten and Orten, '43; Metcoff, Favour and Stare, '45) or a qualitatively incomplete protein (Hogan, Powell and Guerrant, '41; Harris, Neuberger and Sanger, '43; Albanese, Holt, Kajdi and Frankston, '43; and Orten, Bourque and Orten, '45) will result in the development of a mild to moderate chronic anemia. Other investigations have shown the need of an adequate quantity of dietary protein for satisfactory hemopoiesis in the dog (Sturgis and Farrar, '35; Hahn and Whipple, '39) and in man (Bethel, '36; Leverton, McMillan and Peters, '44).

The problem of the comparative value of different commonly consumed dietary proteins for hematopoiesis is raised by the foregoing studies. The scanty information on this subject in the literature is open to criticism because of short feeding periods and errors introduced by the utilization of stored or tissue protein. In order to completely control this factor, it

[1] Aided by a grant from the Griffith Laboratories, Chicago, Illinois.
Preliminary reports appeared in the Federation Proceedings, vol. 2, p. 67 ('43) and vol. 3, p. 95 ('44).

appears necessary that the protein to be evaluated must be the only protein the test animal has ingested for a very long period of time, preferably since weaning. The present study was undertaken with this object in mind and the rat was selected as the test animal for obvious practical reasons.

The proteins lactalbumin, casein, dried skim milk, dried beef blood, and a mixture of dried skim milk and dried beef blood were used. These were fed at both an 18% and a 2.8% protein level to groups of weanling rats. The low-protein level was used to exaggerate possible slight differences between proteins which might not be evident at a higher level. Body weights were followed simultaneously so that a comparison of the value of the proteins for somatic growth as well as for hemoglobin formation was possible. After the animals had been on the experiment for approximately 200 days the hematopoietic value of the various proteins was determined by another technique, that of recovery from a standardized, hemorrhagic anemia.

PROCEDURE

Male and female rats of the Connecticut Agricultural Experiment Station strain, weighing from 40 to 50 gm at weaning, were used. They were housed in individual, wide-mesh bottom cages. The litters were distributed into the various groups, using 10 to 14 rats in each group. The composition of the diets is given in table 1. The protein content of the various preparations was determined by nitrogen analysis of dried, ash-free samples. The following N-conversion factors were used: dried skim milk, 6.38; dried beef blood, 6.25; dried skim milk-beef blood mixture, 6.34. In the case of lactalbumin and casein, the moisture and ash contents of the samples employed were determined and were allowed for in computing the amounts of these two proteins used in the diets. The amount of dextrin in the diet was altered to allow for variations in the amounts of the protein containing materials used, as shown in table 1. The ratio of dried skim milk to dried beef blood solids in the mixture employed was 7 to 3.

Body weights were recorded weekly and hemoglobin determinations were made at the intervals indicated in table 2. An acid-hematin photoelectric method, repeatedly checked against samples of blood, the hemoglobin content of which had been determined by the oxygen-capacity procedure, was used. Erythrocyte counts were also made when the animals were 120 days of age.

TABLE 1

Percentage composition of diets.

DIET	PROTEIN SOURCE	DEXTRIN	SUCROSE	CRISCO	WESSON'S SALT MIXTURE
1. Diets containing 18% protein					
Lactalbumin	22.5	36.5	10	27	4
Casein	22.5	36 5	10	27	4
Blood solids-milk solids mixture	32.9	26.1	10	27	4
Skim milk solids	50.6	8.4	10	27	4
Beef blood solids	20.0	39.0	10	27	4
2. Diets containing 2.8% protein					
Lactalbumin	3 5	55 5	10	27	4
Casein	3.5	55.5	10	27	4
Blood solids-milk solids mixture	5.1	53.9	10	27	4
Skim milk solids	7.9	51.1	10	27	4
Beef blood solids	3.1	55.9	10	27	4

Vitamin supplements: "Ryzamin B" (no. 2) — 200 mg daily.
Wilson's liver ext. "B" — 200 mg daily.
Haliver oil with viosterol — 3 drops twice weekly.

When the animals fed the 18% protein level and the surviving rats fed 2.8% protein as lactalbumin had been on the experiment for approximately 200 days, the value of the various proteins for hemoglobin formation in response to hemorrhage was determined in the following manner. The hemoglobin content of the blood of the rat was first determined. Then 30% of the calculated blood volume (2.0 ml blood per 100 gm body weight) was removed with the aid of gentle massage from the warmed, oiled tail. An equal volume

of 0.9% physiological saline was immediately injected intraperitoneally for fluid replacement. Hemoglobin determinations were then made 24 hours later and at regular intervals thereafter until the concentration reached the initial, prehemorrhage level. The days required for the hemoglobin level to return to the original value were thus determined. The food consumption of the animal was carefully measured during the period of recovery from the hemorrhagic anemia.

Using the data obtained above the following values were calculated in the manner indicated:

(1) Mg Hb removed = ml blood removed × mg Hb per ml.

(2) $$\text{Mg Hb formed per day} = \frac{\text{Mg Hb removed}}{\text{Days to regain initial Hb value}}$$

(3) $$\text{Mg Hb formed per day per 100 gm body wt.} = \frac{\text{Mg Hb formed per day}}{\text{Body weight in 100 gm}}$$

(4) $$\text{Mg Hb formed per day per gm dietary protein consumed} = \frac{\text{Mg Hb formed per day}}{\text{Gm protein ingested daily}}$$

The value of each protein for hemoglobin regeneration was determined two or three times on each animal of a given group and the results were averaged. Successive hemorrhages had no consistent effect on the ability of the animal to form new hemoglobin.

RESULTS

As is shown by the averaged body weight data given in table 2, lactalbumin and casein fed at an 18% protein level gave a somewhat better rate of growth in male rats than did either skim milk solids or the mixture of skim milk solids and beef blood solids. In contrast, beef blood solids alone, although fed at the same protein level, supported only a very slight increase in body weight. The same differences in growth rate were found in the female rats of the various groups (data not included in table 2).

Of the male animals fed the low level of protein (2.8%, table 2), those receiving lactalbumin were definitely better off than those receiving casein or protein from the other sources. As was to be expected, the rate of growth was only slight in

any case, but all of the lactalbumin-fed rats survived a 180-day experimental period whereas nearly all of the animals receiving casein or the other sources of protein succumbed. The same was true of the female rats in the various groups.

The averaged hemoglobin data, given in table 3, indicate that the various proteins, with the possible exception of blood solids, fed at an 18% protein level, are of about equal value in maintaining a normal hemoglobin level in the growing rat.

TABLE 2

Average body weights (in gm) of groups of male rats fed various proteins.

PROTEIN	NO. RATS	DAYS OF AGE						
		24	40	55	77	90	120	200
		1. 18% Protein level						
Lactalbumin	7	42	131	215	339	394	431	552
Casein	8	43	144	234	389	437	506	572
Blood solids-milk solids mixture	8	44	92	145	252	254	301	415
Skim milk solids	5	43	80	117	188	224	305	438
Beef blood solids	6	44	36	39	47	49	61	62
		2. 2.8% Protein level						
Lactalbumin	5	44	47	49	55	59	74	80 [1]
Casein	6	43	42	40	38	37	..	..
Blood solids-milk solids mixture	6	46	42	41	39	37	..	
Skim milk solids	5	45	42	40	44	44	42	42 [1,2]
Beef blood solids	6	45	36	36	37	34	35	35 [1,2]

[1] The rats in this group were 180 days of age.

[2] Data are from one surviving rat.

Slightly lower values were usually found in the animals fed blood solids. The averaged erythrocyte data substantiate this general trend, as do the results obtained in the rats fed the various proteins at a 2.8% protein level, although there are considerable variations in the results in the latter instance. A general parallelism between the value of the various proteins for supporting somatic growth and for hemoglobin formation is suggested by these results.

The data obtained in the studies employing the hemorrhagic anemia technique, however, demonstrate more clearly a difference between the hemopoietic value of blood solids and the other proteins tested. It is evident from the average values given in table 4 that there is no significant difference between lactalbumin, casein, milk solids, and the mixture of beef blood and milk solids in their capacity to support hemoglobin regeneration after hemorrhage. The days after hemorrhage

TABLE 3

Group average hemoglobin and erythrocyte data.

PROTEIN	NO. RATS	HEMOGLOBIN — GM/100 ML Days of age				ERYTHROCYTE COUNT AT 120 DAYS M/mm³
		26	77	90	120	
		1. 18% Protein				
Lactalbumin	13	11.3	16.0	16.5	16.4	7.76
Casein	12	10.8	14.6	15.1	15.4	7.73
Blood solids-milk solids mixture	14	11.7	16.1	16.1	15.7	7.37
Skim milk solids	11	12.7	15.5	15.6	15.1	7.55
Beef blood solids	13	13.6	15.1	15.4	15.2	6.94
		2. 2.8% Protein				
Lactalbumin	12	11.0	12.1	12.6	10.7	5.54
Casein	10	10.6	7.0	6.5	...	4.34 [1]
Blood solids-milk solids mixture	14	13.2	11.2	10.8	8.8	3.79
Skim milk solids	10	11.7	11.2	12.6	8.5	5.67
Beef blood solids	10	13.1	12.2	11.8	10.5	4.54

[1] Erythrocyte counts at approximately 77 days of age.

required for the hemoglobin level to regain the initial value and the mg hemoglobin regenerated per rat, per 100 gm body weight per day, and per gm dietary protein consumed per day are all similar. Paradoxically, however, in the rats fed beef blood solids as the source of protein (18% level) a consistently longer period was required for the hemoglobin level to return to normal and less hemoglobin was regenerated per rat per day, per 100 gm body weight per day, and per gm dietary protein consumed per day. The differences between the values

obtained in the animals fed blood solids and those fed the other proteins have been found to be statistically significant. Using the group fed casein for comparison, the following "significance ratios" were found: (1) mg hemoglobin per 100 gm body weight = 3.0; (2) mg hemoglobin per gm dietary protein = 10.3.

No consistent change in body weight of any of the animals occurred during the period of recovery from hemorrhage.

TABLE 4

Effect of various proteins on hemoglobin formation in hemorrhagic anemia.

PROTEIN	NO. RATS	AV. BODY WT.	AV. PROTEIN DAILY	HB:[1] DAYS TO INITIAL	MG HEMOGLOBIN FORMED PER DAY [1]		
					Per rat	Per 100 gm body wt.	Per gm protein
18% Level		*gm*	*gm*				
Casein	10	531	2.1	15 ± 0.4	107.1 ± 7.1	20.0 ± 0.6	48.3 ± 3.0
Lactalbumin	12	523	2.0	17 ± 0.7	95.3 ± 4.5	18.1 ± 0.8	49.5 ± 1.9
Blood solids-milk solids mixture	12	477	1.9	16 ± 0.5	102.5 ± 4.4	21.9 ± 0.9	52.7 ± 2.1
Skim milk solids	7	358	1.7	15 ± 0.6	76.3 ± 5.7	21.2 ± 0.7	45.1 ± 3.3
Beef blood solids	10	93	1.1	21 ± 1.0	15.1 ± 1.3	16.4 ± 1.1	14.8 ± 1.2
2.8% Level							
Lactalbumin	6	88	0.13	28 ± 2.4	9.3 ± 1.7	10.0 ± 1.4	66.7 ± 6.8

[1] The values given are group averages with the probable errors of the means.

Of considerable interest also are the data obtained on the surviving rats fed lactalbumin at the low level (2.8% protein). As the averaged data show, a much longer time was required for the hemoglobin level to attain the initial value and much less hemoglobin was regenerated per rat per day and per 100 gm body weight per day. However, when calculated as mg hemoglobin regenerated per gm dietary protein consumed per day a strikingly different circumstance is seen — the mg hemoglobin regenerated per gm protein is greater than that formed when 18% protein as lactalbumin or other sources is provided. These differences have been found to be

statistically significant. Again using the group of rats fed 18% protein as casein for comparison, the following "significance ratios" were obtained: (1) mg hemoglobin regenerated per 100 gm body weight = 6.5; (2) mg hemoglobin per gm dietary protein ingested = 2.6. These observations are interpreted as further evidence that if the protein intake is restricted, hemoglobin formation has a "high priority" for the available protein in the organism and therefore hemoglobin synthesis takes precedence over the formation of general body tissue protein. This interpretation is in accord with that drawn from data obtained on dogs by Whipple and his associates (Whipple, '42).

DISCUSSION

The foregoing data emphasize the importance of both the quality and quantity of dietary protein for hemoglobin formation in the rat. The results indicate that, for the proteins studied, those proteins which are qualitatively best constituted for supporting somatic growth are also most effective for hemoglobin formation. Such a thesis is borne out by a variety of other evidence. If rats are fed a synthetic diet containing human or beef globin as the protein, poor growth occurs and an anemia develops (Orten, Bourque and Orten, '45). If the chief qualitative deficiency in the amino acid composition of globin is remedied, by the addition of isoleucine, an increased rate of growth occurs and the anemia is corrected. The same is true if the dietary intake of tryptophane (Albanese et al., '43) or lysine (Hogan et al., '41; Harris et al., '43) is inadequate.

The finding that the feeding of beef blood protein results in the maintenance of a somewhat lower hemoglobin level and slower rate of hemoglobin regeneration after hemorrhage than was found when lactalbumin or casein was fed was rather unexpected. It would appear probable that beef hemoglobin, the principle protein, should be superior since its amino acid composition should be similar to that of rat hemoglobin. Also, the work of Whipple and his associates (Miller,

Robscheit-Robbins and Whipple, '45) indicates an effective utilization of parenterally administered hemoglobin in forming new hemoglobin in the dog. The latter observations are complicated, however, by the fact that additional protein, or the required additional amino acids, could have been obtained from the dog's own tissue protein.

Two explanations of the paradoxical failure of beef blood protein to support satisfactory hemopoiesis in the rat seem possible. One, that rat hemoglobin differs widely in its amino acid composition from beef hemoglobin. Such a possibility seems rather unlikely, however, since although no data on the amino acid composition of rat hemoglobin are available, the values obtained on the hemoglobins of certain other species (Block, '34) indicate some general similarity rather than a wide dissimilarity.

Another, perhaps more logical explanation, is that hemoglobin is synthesized in the organism through some intermediate protein which contains, among other "essential" amino acids, isoleucine. Hence the feeding of the protein, hemoglobin, which is deficient in isoleucine, would result in an unsatisfactory rate of synthesis of the postulated intermediate protein and, in turn as was found, an unsatisfactory synthesis of hemoglobin. Such an hypothesis would also be in accord with the observations that other qualitatively deficient proteins are unsatisfactory for hemopoiesis and that proteins best suited for supporting satisfactory somatic growth are also best suited for hemoglobin formation.

The finding that, whereas dried beef blood is unsatisfactory for supporting somatic growth and hemoglobin formation, the mixture of dried beef blood and dried skim milk supported excellent body growth and hemopoiesis supports the thesis that the inadequacy of dried beef blood protein is due to a deficiency of one or perhaps more amino acids (Orten, Bourque and Orten, '45). Undoubtedly, the protein of dried skim milk supplies the principal lacking amino acid, isoleucine, in amounts sufficient to bring about a satisfactory rate of growth and hemopoiesis.

SUMMARY

Five sources of protein have been studied with respect to their ability (1) to maintain normal hemoglobin levels in the growing rat and (2) to support hemoglobin regeneration after hemorrhage in the adult rat. The various preparations were fed to weanling animals at both 18% and 2.8% levels of protein for an experimental period of approximately 200 days.

At the 18% protein level, casein, lactalbumin, dried skim milk, and a mixture of dried skim milk and dried beef blood proved to be of about the same value, both for hemoglobin maintenance in the growing rat and for hemoglobin regeneration in the adult animal. On the other hand, dried beef blood protein was inferior, particularly for hemoglobin regeneration after hemorrhage.

There appeared to be a general parallelism between the hemopoietic value of the protein and its ability to support somatic growth.

At the 2.8% level of the various proteins, a mild to moderate anemia developed in all cases. However, only those animals receiving lactalbumin survived for the entire experimental period. In these animals, there was a significantly decreased rate of hemoglobin regeneration following hemorrhage. However, there was a very efficient utilization for hemoglobin regeneration of the limited amount of protein consumed.

These observations emphasize the importance of both the quality and quantity of dietary protein for hemopoiesis in the rat.

ACKNOWLEDGMENT

Sincere appreciation is expressed to Mr. F. W. Griffith and Dr. Lloyd A. Hall of the Griffith Laboratories for their financial support of this study and for supplying the dried beef blood, the dried skim milk, and the commercial mixture of dried blood-skim milk (U. S. Patent nos. 2, 155, 417, and 2, 251, 334) used in this investigation.

LITERATURE CITED

ALBANESE, A. A., L. E. HOLT, C. N. KAJDI AND J. E. FRANKSTON 1943 Observations on tryptophane deficiency in rats. J. Biol. Chem., vol. 148, p. 299.

BETHELL, F. H. 1936 The blood changes in normal pregnancy and their relation to the iron and protein supplied by the diet. J. Am. Med. Assoc., vol. 107, p. 564.

BLOCK, R. J. 1934 The basic amino acids of three crystalline mammalian hemoglobins. J. Biol. Chem., vol. 105, p. 663.

HAHN, P. F., AND G. H. WHIPPLE 1939 Hemoglobin production in anemia limited by low protein intake. J. Exp. Med., vol. 69, p. 315.

HARRIS, H. A., A. NEUBERGER AND F. SANGER 1943 Lysine deficiency in young rats. Biochem. J., vol. 37, p. 508.

HOGAN, A. G., E. L. POWELL AND R. E. GUERRANT 1941 Anemia from lysine deficiency deaminized casein. J. Biol. Chem., vol. 137, p. 41.

LEVERTON, R. M., T. J. MCMILLAN AND M. PETERS 1944 Blood regeneration in women blood donors. I. The effect of generous amounts of meat and milk in the diet. J. Am. Dietetic Assoc., vol. 20, p. 747.

METCOFF, J., C. B. FAVOUR AND F. J. STARE 1945 Plasma protein and hemoglobin in the protein-deficient rat — A three dimensional study. J. Clin. Invest., vol. 24, p. 82.

MILLER, L. L., F. S. ROBSCHEIT-ROBBINS AND G. H. WHIPPLE 1945 Hemoglobin and plasma protein. Their relation to internal body protein metabolism. J. Exp. Med., vol. 81, p. 405.

ORTEN, A. U., AND J. M. ORTEN 1943 The role of dietary protein in hemoglobin formation. J. Nutrition, vol. 26, p. 21.

ORTEN, J. M., J. E. BOURQUE AND A. U. ORTEN 1945 The inability of human or beef globin to support normal hematopoiesis in the rat without added isoleucine. J. Biol. Chem., vol. 160, p. 435.

STURGIS, C. C., AND G. E. FARRAR 1935 Hemoglobin regeneration in the chronic hemorrhagic anemia of dogs. I. The effect of iron and protein feeding. J. Exp. Med., vol. 62, p. 457.

WHIPPLE, G. H. 1942 Hemoglobin and plasma proteins: Their production, utilization, and interrelation. Am. J. Med. Sci., vol. 203, p. 477.

INDEX

ACTIVITY, provitamin A, relative, of carotene when introduced orally and parenterally in rat. Studies on carotenoid metabolism. VI. 299
Adult human subjects, calcium metabolism and calcium requirements of, variability in 407
—— —— —— —— —— of, effect of citrate ion on 423
Albino rat, growing, Relation of fat to economy of food utilization. I. 203
—— —— mature, Relation of fat to economy of food utilization. II. 213
ALLISON, JAMES B., ROBERT D. SEELEY, JACK H. BROWN AND JOHN A. ANDERSON. The evaluation of proteins in hypoproteinemic dogs 237
Alpha-tocopherol and essential unsaturated fat acids, interrelation of. Covitamin studies. V. 699
Amino acid excretion and nitrogen balance, intravenously administered nitrogen on, relative efficiency of different forms of 581
—— acids, essential, biological value of proteins in relation to, which they contain. III. Comparison of proteins with mixtures of the amino acids 715
—— —— —— —— —

—— —— —— LAURA C. YDSE, MARY M. AYRES AND RICHARD H. BARNES. The use of mice for the measurement of the growth promoting quality of proteins ... 23
BOUTWELL, ROSWELL K., ROBERT P. GEYER, ANDREW W. HALVERSON AND EDWIN B. HART. The availability of wheat bran phosphorus for the rat ... 193
Bovine, young, vitamin A, ascorbic acid and spinal fluid pressure relationships in ... 229
Bran phosphorus, wheat, availability of, for rat ... 193
BREITER, HERTA. See McLean, Lewis, Jensen, Hathaway, Breiter and Holmes ... 127
BRIGGS, GEORGE M. Nicotinic acid deficiency in turkey poults and the occurrence of perosis ... 79
Bronze poults, failure of feather pigmentation in, due to lysine deficiency ... 387
BROWN, EDNA. See Murlin, Edwards, Hawley and Clark ... 533
—— JACK H. See Allison, Seeley, Brown and Anderson ... 237
Butter fat or corn oil, lactose-dried milk ration containing, rats fed, growth and food preference of ... 321

CALCIFICATION in poults, relative efficiency of vitamin D_3 and cod liver oil in promoting, influence of sources of phosphorus on ... 599
Calcium, dietary, level of, effect of variations in, upon the growth of young rats receiving atabrine ... 647
—— —— retention of, by growing rats, effect of different grades of cocoa upon ... 377
—— metabolism and calcium requirements, variability in, of adult human subjects ... 407
—— —— of adult human subjects, effect of citrate ion on ... 423
—— phosphorus and vitamin D_3, influence of, upon fat content of skeleton in growing pigs ... 565
—— requirement, further studies on, of preschool children ... 127
—— requirements and calcium metabolism, variability in, of adult human subjects ... 407
Calories, restricted, growth rate with, and on injection of the growth hormone. Studies on the comparative nutritive value of fats. VII. ... 737
Canned foods, nutritive value of. XVII. Pyridoxine, biotin and "folic acid" ... 347
Cannon method and rat growth method, evaluation by. Studies on nutritive value of fish proteins. I. ... 175
Carbohydrate, protein and fat in diet, effect of amount of, on incidence and extent of carious lesions. Dental caries in the cotton rat. VI. ... 439
Caries, dental, in cotton rat. VI. The effect of the amount of protein, fat and carbohydrate in the diet on the incidence and extent of carious lesions ... 439
Carious lesions, incidence and extent of, protein, fat and carbohydrate in diet on, effect of amount of. Dental caries in the cotton rat. VI. ... 439
CARLSON, ANTON J., AND FREDERICK HOELZEL. Apparent prolongation of the life span of rats by intermittent fasting ... 363
Carotene and vitamin A, utilization of, influence of choline and of trypan blue upon, for liver storage of vitamin A ... 333
Carotene content of Cuban foods ... 463
Carotenoid metabolism, studies on. VI. Relative provitamin A activity of carotene when introduced orally and parenterally in rat ... 299
CHELDELIN, VERNON H. See Sarett, Bennett, Riggs and Cheldelin ... 755
Chick, availability of cystine and methionine for, influence of autoclaving soybean oil meal on ... 449
—— diet of, protein supplements in, nutritional significance of ... 485
—— plasma fibrinogen in, influence of diet on ... 509
Chicken, energy and gaseous metabolism of, from hatch to maturity as affected by temperature ... 35
Children, preschool, calcium requirement of, further studies on ... 127
—— —— nitrogen metabolism of ... 665
—— —— phosphorus metabolism of ... 657
Choline and trypan blue, influence of, upon utilization of carotene and vitamin A for liver storage of vitamin A ... 333
—— deficiency, development of hepatic cirrhosis due to, significance of fatty infiltration in ... 141
—— —— hemorrhagic kidneys due to, occurrence of, factors affecting ... 621
Cirrhosis, hepatic, due to choline deficiency, development of, significance of fatty infiltration in ... 141
Citrate ion, effect of, on calcium metabolism of adult human subjects ... 423
CLAGETT, DOROTHY D. See Lew, Clagett, Meyer and Hathaway ... 665
CLARK, LELAND C. See Murlin, Edwards, Hawley and Clark ... 533
—— —— —— —

Copper, dietary iron and cobalt, some interrelationships of, in metabolism 609
Corn germ, wheat germ, and some other protein foods, proteins of, comparative growth-promoting value of, of plant and animal origin 675
—— oil or butter fat, lactose-dried milk ration containing, rats fed, growth and food preference of 321
Cotton rat, dental caries in. VI. The effect of the amount of protein, fat and carbohydrate in the diet on the incidence and extent of carious lesions 439
Covitamin studies. V. The interrelation of alpha-tocopherol and essential unsaturated fat acids 699
Cow and ewe, colostrum and milk of, thiamine, riboflavin, nicotinic acid and pantothenic acid content of 51
Cox, Warren M., Jr., and Arthur J. Mueller. The relative efficiency of different forms of intravenously administered nitrogen on nitrogen balance and amino acid excretion 581
—— —— —— —— See Mueller and Cox 249
Crockett, Mary E. See Deuel, Hendrick and Crockett 737
—— —— —— —— —— —— Movitt, Crockett, Smyth and Winzler 747
Crops, vegetable, in relation to soil fertility. IV. Nutritional values of New Zealand spinach 59
Cuban foods, carotene content of 463
Cunha, Tony J. See Heinemann, Ensminger, Cunha and McCulloch 107
Cystine and methionine, availability of, influence of autoclaving soybean oil meal on, for chick 449

Dam, Henrick. See Field and Dam 509
Darnell, A. L. See Pearson and Darnell 51
Day, Harry G., Khalil G. Wakim, William H. Zimmerman and Leland S. McClung. Nutritional Nutritional status of rats on milk diets containing succinylsulfathiazole 355
Deficiencies, pyridoxine and pantothenic acid, study of, in monkey (Macaca mulatta) 685
Deficiency, choline, development of hepatic cirrhosis due to, significance of fatty infiltration in 141
—— —— hemorrhagic kidneys due to, occurrence of, factors affecting 621
—— lysine, failure of feather pigmentation in bronze poults due to 387
—— nicotinic acid, in turkey poults and occurrence of perosis 79
—— pantothenic acid, and reproduction in rat 497
Dental caries in the cotton rat. VI. The effect of the amount of protein, fat and carbohydrate in the diet on the incidence and extent of carious lesions 439
Determination of metabolic fecal nitrogen and protein digestibility 13
Deuel, Harry J., Jr., Cornelia Hendrick and Mary E. Crockett. Studies on the comparative nutritive value of fats. VII. Growth rate with restricted calories and on injection of the growth hormone 737
—— —— —— —— —— —— Eli Movitt, Mary E. Crockett, Irene M. Smyth and Richard J. Winzler. Studies on the comparative nutritive value of fats. VIII. The failure of ether extraction to lower the nutritive value of skimmed milk powder in diets containing various vegetable fats 747
—— —— —— —— Mary C. Hrubetz, Cornelia H. Johnston, Hilda S. Rollman and Ernest Geiger. Studies on the nutritive value of fish proteins. II. The use of mackerel protein in the bioassay test for vitamin A 187
—— —— —— —— —— —— Richard J. Winzler, Ernest Geiger and George Schnakenberg. Studies on the nutritive value of fish proteins. I. Evaluation by the rat growth method and by the Cannon method 175
—— —— —— —— See Sexton, Mehl and Deuel 299
Diet(s), changes in, effect of, on volume and composition of rat milk 249
—— containing various vegetable fats, skimmed milk powder in, nutritive value of, failure of ether extraction to lower. Studies on the comparative nutritive value of fats. VIII. 747
—— influence of, on plasma fibrinogen in chick 509
—— milk, rats on, nutritional status of, containing succinylsulfathiazole 355
—— of chick, animal protein supplements in, nutritional significance of 485
—— protein, fat and carbohydrate in, effect of amount of, on incidence and extent of carious lesions. Dental caries in the cotton rat. VI. 439
—— self selection of. I. Selection of purified components 397
Dietary calcium, level of, effect of variations in, upon the growth of young rats receiving atabrine 647
—— —— retention of, effect of different grades of cocoa upon, by growing rats 377
—— iron, copper and cobalt, some interrelationships of, in metabolism 609
—— niacin and tryptophane requirement, factors affecting, of growing rat 85
—— proteins, certain, comparative value of, for hemopoiesis in rat 765
Digestibility, protein, and metabolic fecal nitrogen, determination of 13
Dogs, hypoproteinemic, proteins in, evaluation of 237
Dubin, Isadore N. See Handler and Dubin 141

Edwards Leslie E. See Murlin, Edwards, Fried and Szymanski 715
—— —— —— —— —— —— Hawley and Clark 533
—— —— —— —— —— —— —— —— 555
Effect of changes in diet on the volume and composition of rat milk 249
—— —— different grades of cocoa upon the retention of dietary calcium by growing rat 377

—— —— the amount of protein, fat and carbohydrate in the diet on the incidence and extent of carious lesions. Dental caries in the cotton rat. VI. 439
—— —— —— citrate ion on the calcium metabolism of adult human subjects 423
—— —— variations in the level of dietary calcium upon the growth of young rats receiving atabrine 647
Effects of added vitamin A on the conjunctiva and the level of vitamin A in the blood 471
Efficiency of swimmers, observations on, as related to some changes in pre-exercise nutriment 525
—— relative, of different forms of intravenously administered nitrogen balance and amino acid excretion 581
—— —— —— vitamin D_3 and cod liver oil, influence of sources of phosphorus on, in promoting calcification in poults 599
Egg and soy protein, biological values illustrated by supplementing, interconvertibility of, with essential amino acids. Biological value of proteins in relation to essential amino acids which they contain. II. 555
ELLIOTT, RALPH F. See Forbes, Swift, Elliott and James 203
—— —— —— —— —— —— —— —— 213
ELVEHJEM, CONRAD A. See Ives, Pollard, Elvehjem and Strong 347
—— —— —— —— Krehl, Sarma, Teply and Elvehjem 85
—— —— —— —— McCall, Waisman, and Jones 685
—— —— —— —— Schweigert, Shaw, Zepplin and Elvehjem 439
Endogenous nitrogen of man. Biological value of proteins in relation to the essential amino acids which they contain. I. 533
Energy and gaseous metabolism of the chicken from hatch to maturity as affected by temperature 35
ENSMINGER, MARION E. See Heinemann, Ensminger, Cunha and McCulloch 107
Ether extraction, failure of, to lower the nutritive value of skimmed milk powder in diets containing various vegetable fats. Studies on the comparative nutritive value of fats. VIII. 747
Evaluation by the rat growth method and by the Cannon method. Studies on the nutritive value of fish proteins. I. 175
—— of proteins in hypoproteinemic dogs 237
EVANS, HERBERT M. See Nelson and Evans 497
EVANS, ROBERT J., AND JAMES McGINNIS. The influence of autoclaving soybean oil meal on the availability of cystine and methionine for the chick 449
Evaporated milk, market milk, and powdered whole milk, vitamin C content of 161
Ewe and cow, colostrum and milk of, thiamine, riboflavin, nicotinic acid and pantothenic acid content of 51
Excretion, amino acid, and nitrogen balance, intravenously administered nitrogen on, relative efficiency of different forms of 581
—— urinary, of thiamine, four methods for studying, comparison of 635
Extraction, ether, failure of, to lower the nutritive value of skimmed milk powder in diets containing various vegetable fats. Studies on the comparative nutritive value of fats. VIII. 747
Eye condition, length of life, growth and tooth structure, relation of vitamin A intake to 67

FACTORS affecting the dietary niacin and tryptophane requirement of the growing rat 85
—— —— occurrence of hemorrhagic kidneys due to choline deficiency 621
Failure of ether extraction to lower the nutritive value of skimmed milk powder in diets containing various vegetable fats. Studies on the comparative nutritive value of fats. VIII. 747
—— —— feather pigmentation in bronze poults due to lysine deficiency 387
Fasting, intermittent, life span of rats by, apparent prolongation of 363
Fat(s) acid, essential unsaturated, and alpha-tocopherol, interrelation of. Covitamin studies. V. 699
—— butter, or corn oil, lactose-dried milk ration containing, rats fed, growth and food preference of 321
—— comparative nutritive value of, studies on. VIII. Failure of ether extraction to lower the nutritive value of skimmed milk powder in diets containing various vegetable fats 747
—— —— —— —— —— —— —— —

Fibrinogen, plasma, in chick, influence of diet on ... 509
FIELD, JOHN B., AND HENRIK DAM. Influence of diet on plasma fibrinogen in the chick ... 509
Fish proteins, nutritive value of, studies on. I. Evaluation by rat growth method and by Cannon method ... 175
—— II. The use of mackerel protein in the bioassay test for vitamin A ... 187
Fluid pressure, spinal, vitamin A and ascorbic acid relationships in young bovine ... 229
"Folic acid," pyridoxine and biotin. Nutritive value of canned foods. XVII. ... 347
Food(s), canned, nutritive value of. XVII. Pyridoxine, biotin and "folic acid" ... 347
—— Cuban, carotene content of ... 463
—— preference and growth of rats fed a lactose-dried milk ration containing butter fat or corn oil ... 321
—— restaurant, thiamine, riboflavin, nicotinic acid, pantothenic acid content of ... 755
—— some other protein, wheat germ, and corn germ of plant and animal origin, proteins of, comparative growth-promoting value of ... 675
—— utilization, economy of, relation of fat to. I. By the growing albino rat ... 203
—— II. By the mature albino rat ... 213
FORBES, ERNEST B., RAYMOND W. SWIFT, RALPH F. ELLIOTT AND WILLIAM H. JAMES. Relation of fat to economy of food utilization. I. By the growing albino rat ... 203
—— II. By the mature albino rat ... 213
FRIED, SERAPHINE. See Murlin, Edwards, Fried and Szymanski ... 715
—— —— —— —— —— Hawley and Clark ... 533
FRITZ, JAMES C., JOHN H. HOOPER, JOHN L. HALPIN AND HOWARD P. MOORE. Failure of feather pigmentation in bronze poults due to lysine deficiency ... 387
FUENTES, CESAR. See Angulo, Fuentes and Johnson ... 463
Further studies on the calcium requirement of preschool children ... 127

GASEOUS and energy metabolism of chicken from hatch to maturity as affected by temperature ... 35
GEIGER, ERNEST. See Deuel, Hrubetz, Johnston, Rollman and Geiger ... 187
—— —— —— —— —— Winkler, Geiger and Schnakenberg ... 175
Germ, corn, wheat germ and some other protein foods of plant and animal origin, proteins of, comparative growth-promoting value of ... 675
GEYER, ROBERT P. See Boutwell, Geyer, Halverson and Hart ... 193
GIFFT, HELEN H., AND HAZEL M. HAUCK. A comparison of four methods for studying the urinary excretion of thiamine ... 635
GILL, DOROTHY. See Murlin, Edwards, Fried and Szymanski ... 715
—— —— —— —— —— Hawley and Clark ... 533
GOFF, H. R. See Wittwer and Goff ... 59
Growing albino rat. Relation of fat to economy of food utilization. I. ... 203
—— pigs, skeleton in, fat content of, influence of phosphorus, calcium and vitamin D_3 upon ... 565
—— rats, retention of dietary calcium by, effect of different grades of cocoa upon ... 377
Growth and food preference of rats fed a lactose-dried milk ration containing butter fat or corn oil ... 321
—— hormone, injection of, growth rate with restricted calories and on. Studies on the comparative nutritive value of fats. VII. ... 737
—— length of life, tooth structure and eye condition, relation of vitamin A intake to ... 67
—— of young rats receiving atabrine, dietary calcium upon, effect of variations in the level of ... 647
—— promoting quality, use of mice for measurement of, of proteins ... 23
—— —— value, comparative, of the proteins of wheat germ, corn germ, and of some other protein foods of plant and animal origin ... 675
—— rat method and Cannon method, evaluation by. Studies on nutritive value of fish proteins. I. ... 175
—— rate with restricted calories and on injection of the growth hormone. Studies on the comparative nutritive value of fats. VII. ... 737

HALDI, JOHN, AND WINFREY WYNN. Observations on efficiency of swimmers as related to some changes in pre-exercise nutriment ... 525
HALPIN, J. L. See Fritz, Hooper, Halpin and Moore ... 387
HALVERSON, ANDREW W. See Boutwell, Geyer, Halverson and Hart ... 193
HAMILTON, TOM S. See Mitchell and Hamilton ... 377
HANDLER, PHILIP. Factors affecting the occurrence of hemorrhagic kidneys due to cholin deficiency ... 621
—— —— AND ISADORE N. DUBIN. The significance of fatty infiltration in the development of hepatic cirrhosis due to choline deficiency ... 141
HARRELL, RUTH F. Mental response to added thiamine ... 283
HARRIS, PHILIP L. See Hove and Harris ... 699
HART, EDWIN B. See Boutwell, Geyer, Halverson and Hart ... 193
HATHAWAY, MILICENT L. See Lew, Clagett, Meyer and Hathaway ... 665
—— —— —— —— McKey, Clark, Meyer and Hathaway ... 657
—— —— —— —— McLean, Lewis, Jensen, Hathaway, Breiter and Holmes ... 127
HAUCK, HAZEL M. See Gifft and Hauck ... 635
HAWLEY, ESTELLE E. See Murlin, Edwards, Hawley and Clark ... 533
—— —— ... 555
HEGSTED, D. M. See Williamson, Hegsted, McKibbin and Stare ... 647

HEINEMANN, WILTON W., MARION E. ENSMINGER, TONY J. CUNHA AND ERNEST C. MCCULLOCH. The relation of the amount of thiamine in the ration of the hog to the thiamine and riboflavin content of the tissue ... 107
Hemopoiesis in rat, certain dietary proteins for, comparative value of ... 765
Hemorrhagic kidneys, occurrence of, factors affecting, due to choline deficiency ... 621
HENDRICK, CORNELIA. See Deuel, Hendrick and Crockett ... 737
—— —— —— —— —— Movitt, Crockett, Smyth and Winzler ... 747
Hepatic cirrhosis due to choline deficiency, development of, significance of fatty infiltration in ... 141
HOELZEL, FREDERICK. See Carlson and Hoelzel ... 363
Hog, ration of, relation of amount of thiamine in, to thiamine and riboflavin content of tissue ... 107
HOLMES, JULIA D. See McLean, Lewis, Jensen, Hathaway, Breiter and Holmes ... 127
HOOPER, J. H. See Fritz, Hooper, Halpin and Moore ... 387
Hormone, growth, injection of, growth rate with restricted calories and on. Studies on the comparative nutritive value of fats. VII. ... 737
HOVE, A. E. H., A. W. THOMAS AND H. C. SHERMAN. Some inter-relationships of dietary iron, copper and cobalt in metabolism ... 609
HOVE, E. L., AND PHILIP L. HARRIS. Covitamin studies. V. The interrelation of alpha-tocopherol and essential unsaturated fat acids ... 699
HRUBETZ, MARY C. See Deuel, Hrubetz, Johnston, Rollman and Geiger ... 187
—— —— —— —— —— Winzler, Geiger and Schnakenberg ... 175
HUGHES, J. S. See Parrish, Shimer and Hughes ... 321
Human subjects, adult, calcium metabolism and calcium requirements of, variability in ... 407
—— —— —— —— —— of, effect of citrate ion on ... 423
Hypoproteinemic dogs, proteins in, evaluation of ... 237

INFILTRATION, fatty, significance of, in development of hepatic cirrhosis due to choline deficiency ... 141
Influence of autoclaving soybean oil meal on the availability of cystine and methionine for the chick ... 449
—— —— choline and of trypan blue upon the utilization of carotene and vitamin A for liver storage of vitamin A ... 333
—— —— diet on plasma fibrinogen in the chick ... 509
—— —— phosphorus, calcium and vitamin D_3 upon the fat content of the skeleton in growing pigs ... 565
—— —— sources of phosphorus on the relative efficiency of vitamin D_3 and cod liver oil in promoting calcification in poults ... 599
Injection of the growth hormone and restricted calories, growth rate with. Studies on the comparative nutritive value of fats. VII. ... 737
Intake, vitamin A, relation of, to length of life, growth, tooth structure and eye condition ... 67
Interconvertibility of biological values illustrated by supplementing egg and soy protein with essential amino acids. Biological value of proteins in relation to the essential amino acids which they contain. II. ... 555
Interrelation of alpha-tocopherol and essential unsaturated fat acids. Covitamin studies. V. ... 699
Intravenously administered nitrogen, different forms of, relative efficiency of, on nitrogen balance and amino acid excretion ... 581
Ion, citrate, effect of, on calcium metabolism of adult human subjects ... 423
Iron, dietary, copper and cobalt, some interrelationships of, in metabolism ... 609
IVES, MARGARET, ANN E. POLLARD, CONRAD A. ELVEHJEM AND FRANK M. STRONG. The nutritive value of canned foods. XVII. Pyridoxine, biotin and "folic acid" ... 347

JAMES, WILLIAM H. See Forbes, Swift, Elliott and James ... 203
—— —— —— —— —— ... 213
JENSEN, ELIZABETH. See McLean, Lewis, Jensen, Hathaway, Breiter and Holmes ... 127
JOHNSON, MARGARITA. See Angulo, Fuentes and Johnson ... 463
JOHNSTON, CORNELIA H. See Deuel, Hrubetz, Johnston, Rollman and Geiger ... 187
—— —— —— —— —— Winzler, Geiger and Schnakenberg ... 175
JONES, D. BREESE, AND KATHERINE D. WIDNESS. The comparative growth-promoting value of the proteins of wheat germ, corn germ, and of some other protein foods of plant and animal origin ... 675
—— EDITH S. See McCall, Waisman, Elvehjem and Jones ... 685

KIDNEYS, hemorrhagic, occurrence of, factors affecting, due to choline deficiency ... 621
KREHL, WILLARD A., P. S. SARMA, LESTER J. TEPLEY AND CONRAD A. ELVEHJEM. Factors affecting the dietary niacin and tryptophane requirement of the growing rat ... 85

LACTOSE-dried milk ration containing butter fat or corn oil, rats fed, growth and food preference of ... 321
Lesions, carious, incidence and extent of, amount of protein, fat and carbohydrate in diet on, effect of. Dental caries in the cotton rat. VI. ... 439
LEW, MARION S., DOROTHY D. CLAGETT, FRIEDA L. MEYER AND MILICENT L. HATHAWAY. Nitrogen metabolism of preschool children ... 665
LEWIS, GLADYS K. See McLean, Lewis, Jensen, Hathaway, Breiter and Holmes ... 127
Liver oil, cod, and vitamin D_3, relative efficiency of, influence of sources of phosphorus on, in promoting calcification in poults ... 599

—— storage of vitamin A, utilization of carotene and vitamin A for, influence of choline and of trypan blue upon 333
Lysine deficiency, failure of feather pigmentation in bronze poults due to 387

McCALL, KEITH B., H. A. WAISMAN, CONRAD A. ELVEHJEM AND EDITH S. JONES. A study of pyridoxine and pantothenic acid deficiencies in the monkey (Macaca mulatta) 685
McCLUNG, LELAND S. See Day, Wakim, Zimmerman and McClung 355
McCULLOCH, ERNEST C. See Heinemann, Ensminger, Cunha and McCulloch 107
McGINNIS, JAMES. See Evans and McGinnis 449
McHARGUE, J. S. See Rapp, Skinner and McHargue 261
—— —— —— —— 273
—— 273
McKEY, BEULA V., MARIE F. CLARK, FRIEDA L. MEYER AND MILICENT L. HATHAWAY. Phosphorus metabolism of preschool children 657
McKIBBIN, J. M. See Williamson, Hegsted, McKibbin and Stare 647
McLEAN, DOROTHY S., GLADYS K. LEWIS, ELIZABETH JENSEN, MILICENT L. HATHAWAY, HERTA BREITER AND JULIA O. HOLMES. Further studies on the calcium requirement of preschool children 127
McMILLAN, THELMA J. See Todhunter and McMillan 573
Mackerel protein, use of, in bioassay test for vitamin A. Studies on nutritive value of fish proteins. II. 187
Man, endogenous nitrogen of. Biological value of proteins in relation to essential amino acids which they contain. I. 533
Market milk, evaporated milk, and powdered whole milk, vitamin C content of 161
MARVEL, J. P. See Patton, Marvel, Petering and Waddell 485
MATTERSON, L. D., H. M. SCOTT AND E. P. SINGSEN. The influence of sources of phosphorus on the relative efficiency of vitamin D_3 and cod liver oil in promoting calcification in poults 599
Mature albino rat. Relation of fat to economy of food utilization. II. 213
Meal, soybean oil, autoclaving, influence of, on availability of cystine and methionine for chick 449
MEHL, JOHN W. See Sexton, Mehl and Deuel 299
Mental response to added thiamine 283
Metabolic fecal nitrogen and protein digestibility, determination of 13
Metabolism, calcium, and calcium requirements, variability in, of adult human subjects 407
—— —— effect of citrate ion on, of adult human subjects 423
—— carotenoid, studies on. VI. Relative provitamin A activity of carotene when introduced orally and parenterally in rat 299
—— dietary iron, copper and cobalt in, some interrelationships of 659
—— energy and gaseous, of chicken from hatch to maturity as affected by temperature 35
—— nitrogen, of preshcool children 665
—— phosphorus, of preschool children 657
Methionine and cystine, availability of, influence of autoclaving soybean oil meal on, for chick 449
MEYER, FRIEDA L. See Lew, Clagett, Meyer and Hathaway 665
—— —— —— —— McKey, Clark, Meyer and Hathaway 657
Mice, use of, for measurement of growth promoting quality of proteins 23
Milk and colostrum, thiamine, riboflavin, nicotinic acid and pantothenic acid content of, of cow and ewe 51
—— diets, rats on, nutritional status of, containing succinylsulfathiazole 355
—— market, evaporated, and powdered whole, vitamin C content of 161
—— powder, skimmed, nutritive value of, failure of ether extraction to lower, in diets containing various vegetable fats. Studies on the comparative nutritive value of fats. VIII. 747
—— rat, volume and composition of, effect of changes in diet on 249
—— ration, lactose-dried, containing butter fat or corn oil, rats fed, growth and food preference of 321
MITCHELL, HAROLD H., AND TOM S. HAMILTON. The effect of different grades of cocoa upon the retention of dietary calcium by growing rats 377
—— —— —— See Steggerda and Mitchell 407
—— —— —— —— —— —— 423
Mixtures of amino acids, comparison of proteins with. Biological value of proteins in relation to the essential amino acids which they contain. III. 715
Monkey (Macaca mulatta), pyridoxine and pantothenic acid deficiencies in, study of 685
MOORE, H. P. See Frits, Hooper, Halpin and Moore 387
—— LANE A. Vitamin A, ascorbic acid and spinal fluid pressure relationships in the young bovine 229
MORGAN, A. LLOYD. See Robertson and Morgan 471
—— AGNES F. See Bentley and Morgan 333
MOVITT, ELI. See Deuel, Hendrick, Movitt, Crockett, Smyth and Winzler 747
MUELLER, ARTHUR J., AND WARREN M. COX, JR. The effect of changes in diet on the volume and composition of rat milk 249
—— —— —— See Cox and Mueller 581
MURLIN, JOHN R., investigator, teacher, colleague. An appreciation 5
—— —— —— LESLIE E. EDWARDS, SERAPHINE FRIED AND THADDEUS A. SZYMANSKI, with the assistance of Elizabeth Nasset, Doris Smith and Dorothy Gill. Biological value of proteins in relation to the essential amino acids which they contain. III. Comparison of proteins with mixtures of the amino acids 715

—— —— —— —— —— —— Estelle E. Hawley and Leland C. Clark. Biological value of proteins in relation to the essential amino acids which they contain. II. Interconvertibility of biological values illustrated by supplementing egg and soy protein with essential amino acids 555

—— —— —— —— —— —

PETERING, H. G. See Patton, Marvel, Petering and Waddell 485
Phosphorus, calcium and vitamin D_3, influence of, upon fat content of skeleton in growing pigs 565
—— metabolism of preschool children 657
—— sources of, influence of, on relative efficiency of vitamin D_3 and cod liver oil in promoting calcification in poults 599
—— wheat bran, availability of, for rat 193
Pigmentation, feather, in bronze poults, failure of, due to lysine deficiency 387
Pigs, growing, skeleton in, fat content of, influence of phosphorus, calcium and vitamin D_3 upon 565
Plant and animal origin, wheat germ, corn germ and some other protein foods of, proteins of, comparative growth-promoting value of 675
Plasma and whole blood of normal rats, ascorbic acid content of, with evidence of sex difference 573
—— fibrinogen in chick, influence of diet on 509
POLLARD, ANN E. See Ives, Pollard, Elvehjem and Strong 347
Poults, bronze, failure of feather pigmentation in, due to lysine deficiency 387
—— calcification in, relative efficiency of vitamin D_3 and cod liver oil in promoting, influence of sources of phosphorus on 599
—— turkey, nicotinic acid deficiency in, and occurrence of perosis 79
Powder, skimmed milk, nutritive value of, failure of ether extraction to lower, in diets containing various vegetable fats. Studies on the comparative nutritive value of fats. VIII. 747
Powdered whole milk, market milk, and evaporated milk, vitamin C content of 161
Pre-exercise nutriment, some changes in, observations on efficiency of swimmers as related to 525
Preschool children, calcium requirement of, further studies on 127
—— —— nitrogen metabolism of 665
—— —— phosphorus metabolism of 657
Pressure, spinal fluid, vitamin A and ascorbic acid relationships in young bovine 229
PRINGLE, EMMA M. See Barrott and Pringle 35
Protein(s), biological value of, in relation to essential amino acids which they contain. III. Comparison of proteins with mixtures of the amino acids 715
—— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— ——
I. Endogenous nitrogen of man 533
—— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— ——
II. Interconvertibility of biological values illustrated by supplementing egg and soy protein with essential amino acids 555
—— certain dietary, comparative value of, for hemopoiesis in rat .. 765
—— comparative growth-promoting value of, of wheat germ, corn germ, and of some other protein foods of plant and animal origin . 675
—— comparison of, with mixtures of the amino acids. Biological value of proteins in relation to the essential amino acids which they contain. III. 715
—— digestibility and metabolic fecal nitrogen, determination of 13
—— evaluation of, in hypoproteinemic dogs 237
—— fat and carbohydrate in diet, effect of amount of, on incidence and extent of carious lesions. Dental caries in the cotton rat. VI. . 439
—— fish, nutritive value of, studies on. I. Evaluation by rat growth method and by Cannon method .. 175
—— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— ——
II. The use of mackerel protein in the bioassay test for vitamin A .. 187
—— foods, some other, wheat germ, and corn germ of plant and animal origin, proteins of, comparative growth-promoting value of 675
—— mackerel, use of, in bioassay test for vitamin A. Studies on nutritive value of fish proteins. II. 187
—— nutritive value of, in tobacco-seed oilmeal 261
—— quality of, growth promoting, use of mice for measurement of .. 23
—— soy, and egg, biological values illustrated by supplementing, interconvertibility of, with essential amino acids. Biological value of proteins in relation to essential amino acids which they contain. II. 555
—— supplements, animal, nutritional significance of, in diet of chick 485
Provitamin A activity relative, of carotene when introduced orally and parenterally in rat. Studies on carotenoid metabolism. VI. . 299
Purified components, selection of. Self selection of diet. I. 397
Pyridoxine and pantothenic acid deficiencies, study of, in monkey (Macaca mulatta) 685
—— biotin and "folic acid." Nutritive value of canned foods. XVII. 347

RAPP, KARL E., J. T. SKINNER AND J. S. MCHARGUE. The nutritive value of the protein in tobacco-seed oilmeal 261
—— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— —— ——
—— tobacco-seed oil 273
Rat(s), availability of wheat bran phosphorus for 193
—— cotton, dental caries in. VI. The effect of the amount of protein, fat and carbohydrate in the diet on the incidence and extent of carious lesions 439
—— growing albino. Relation of fat to economy of food utilization. I. 203
—— —— dietary niacin and tryptophane requirement of, factors affecting 85
—— —— retention of dietary calcium by, effect of different grades of cocoa upon 377
—— growth and food preference of, fed by lactose-dried milk ration containing butter fat or corn oil 321
—— hemopoiesis in, certain dietary proteins for, comparative value of 765

—— —— method and Cannon method, evaluation by. Studies on nutritive value of fish proteins. I. 175
—— mature albino. Relation of fat to economy of food utilization. II. 213
—— milk, volume and composition of, effect of changes in diet on 249
—— normal, whole blood and plasma of, ascorbic acid content of, with evidence of sex difference 573
—— nutritional status of, on milk diets containing succinylsulfathiazole 355
—— pantothenic acid deficiency and reproduction in 497
—— relative provitamin A activity of carotene when introduced orally and parenterally in. Studies on carotenoid metabolism. VI. 299
—— young, growth of, receiving atabrine, dietary calcium upon, effect of variations in the level of 647
Ration, lactose-dried milk, rats fed, growth and food preference of, containing butter fat or corn oil 321
—— of hog, relation of amount of thiamine in, to thiamine and riboflavin content of tissue 107
Relation of fat to economy of food utilization. I. By the growing albino rat 203
—— —— —— —— —— —— —— —— —— —— II. By the mature albino rat 213
—— —— the amount of the thiamine in the ration of the hog to the thiamine and riboflavin content of the tissue 107
—— —— vitamin A intake to length of life, growth, tooth structure and eye condition 67
Relative efficiency of different forms of intravenously administered nitrogen balance and amino acid excretion 581
—— provitamin A activity of carotene when introduced orally and parenterally in the rat. Studies on carotenoid metabolism. VI. 299
Reproduction and pantothenic acid deficiency in rat 497
Requirement, calcium, further studies on, of preschool children 127
—— dietary niacin and tryptophane, factors affecting, of growing rat 85
Requirements, calcium, and calcium metabolism, variability in, of adult human subjects 407
Restaurant foods, thiamine, riboflavin, nicotinic acid, pantothenic acid and ascorbic acid content of 755
Retention of dietary calcium by growing rat, effect of different grades of cocoa upon 377
Riboflavin and thiamine content of tissue, relation of amount of thiamine in ration of hog to 107
—— thiamine, nicotinic acid and pantothenic acid content of colostrum and milk of cow and ewe 51
—— —— —— —— pantothenic acid and ascorbic acid content of restaurant foods 755
Riggs, Thomas R. See Sarett, Bennett, Riggs and Cheldelin 755
Robertson, Elizabeth C., and Alonzo L. Morgan. The effects of added vitamin A on the conjunctiva and the level of vitamin A in the blood 471
Rollman, Hilda S. See Deuel, Hrubetz, Johnston, Rollman and Geiger
Ryer, Helen. See Murlin, Edwards, Hawley and Clark 533
—— —— —— —— —— —— —— —— —— —— 555

Sarett, Herbert P., Margaret J. Bennett, Thomas R. Riggs and Vernon H. Cheldelin. Thiamine, riboflavin, nicotinic acid, and ascorbic acid content of restaurant foods 755
Sarma, P. S. See Krehl, Sarma, Teply and Elvehjem 85
Schnakenberg, George. See Deuel, Hrubetz, Johnston, Winzler, Geiger and Schnakenberg 175
Schweigert, Bernard S., James H. Shaw, Theressa M. Zepplin and Conrad A. Elvehjem. Dental caries in the cotton rat. VI. The effect of the amount of protein, fat and carbohydrate in the diet on the incidence and extent of carious lesions 439
Scott, E. M. Self selection of diet. I. Selection of purified components 397
—— H. M. See Matterson, Scott and Singsen 599
Seeley, Robert D. See Allison, Seeley, Brown and Anderson 237
Self selection of diet. I. Selection of purified components 397
Sex difference, evidence of, whole blood and plasma of normal rats with, ascorbic acid content of 573
Sexton, Edwin L., John W. Mehl and Harry J. Deuel, Jr. Studies on carotenoid metabolism. VI. The relative provitamin A activity of carotene when introduced orally and parenterally in the rat 299
Sharp, Paul F. See Stewart and Sharp 161
Shaw, James H. See Schweigert, Shaw, Zepplin and Elvehjem 439
Sherman, H. C. See Houk, Thomas and Sherman 609
Shimer, E. Roberta. See Parrish, Shimer and Hughes 321
Significance of fatty infiltration in the development of hepatic cirrhosis due to choline deficiency 141
Singsen, E. P. See Matterson, Scott and Singsen 599
Skeleton in growing pigs, fat content of, influence of phosphorus, calcium and vitamin D_3 upon 565
Skimmed milk powder, nutritive value of, failure of ether extraction to lower, in diets containing various vegetable fats. Studies on the comparative nutritive value of fats. VIII. 747
Skinner, J. T. See Rapp, Skinner and McHargue 261
—— —— —— —— —— —— —— —— —— —— 273
Smith, Doris. See Murlin, Edwards, Fried and Szymanski 715
—— —— —— —— —— Hawley and Clark 533
Smyth, Irene M. See Deuel, Hendrick, Movitt, Crockett, Smyth and Winzler 747
Soil fertility, vegetable crops in relation to. IV. Nutritional values of New Zealand spinach 59
Some inter-relationships of dietary iron, copper and cobalt in metabolism 609

Soy protein and egg, biological values illustrated by supplementing, interconvertibility of, with essential amino acids. Biological value of proteins in relation to essential amino acids which they contain. II. 555
Soybean oil meal, autoclaving, influence of, on availability of cystine and methionine for chick 449
Span, life, of rats, apparent prolongation of, by intermittent fasting 363
Spinach, New Zealand, nutritional values of. Vegetable crops in relation to soil fertility 59
Spinal fluid pressure, vitamin A and ascorbic acid relationships in young bovine 299
STARE, F. J. See Williamson, Hegsted, McKibbin and Stare 647
Status, nutritional, of rats on milk diets containing succinylsulfathiazole 355
STEGGERDA, FREDERIC R., AND HAROLD H. MITCHELL. The effect of the citrate ion on the calcium metabolism of adult human subjects 423
—— —— —— —— —— —— Variability in the calcium metabolism and calcium requirements of adult human subjects 407
STEWART, AUBREY P., JR., AND PAUL F. SHARP. Vitamin C content of market milk, evaporated milk and powdered whole milk 161
Storage, liver, of vitamin A, utilization of carotene and vitamin A for, influence of choline and of trypan blue upon 333
STRONG, FRANK M. See Ives, Pollard, Elvehjem and Strong 347
Studies, further, on calcium requirement of preschool children 127
—— on carotenoid metabolism. VI. The relative provitamin A activity of carotene when introduced orally and parenterally in the rat 299
—— —— the comparative nutritive value of fats. VII. Growth rate with restricted calories and on injection of the growth hormone 737
—— —— —— —— —— —— —— VIII. The failure of ether extraction to lower the nutritive value of skimmed milk powder in diets containing various vegetable fats 747
—— —— nutritive value of fish proteins. I. Evaluation by the rat growth method and by the Cannon method 175
—— —— —— —— —— —— —— II. The use of mackerel protein in the bioassay test for vitamin A 187
Study of pyridoxine and pantothenic acid deficiencies in the monkey (Macaca mulatta) 685
Subjects, adult human, calcium metabolism and calcium requirements, variability in 407
—— —— —— —— —— —— of, effect of citrate ion on 423
Succinylsulfathiazole, milk diets containing, nutritional status of rats on 355
Supplements, animal protein, nutritional significance of, in diet of chick 485
SWIFT, RAYMOND W. See Forbes, Swift, Elliott and James 203
—— —— —— —— —— —— —— —— 213
Swimmers, efficiency of, observations on, as related to some changes in pre-exercise nutriment 525
SZYMANSKI, THADDEUS A. See Murlin, Edwards, Fried and Szymanski 715

TEMPERATURE, energy and gaseous metabolism of chicken from hatch to maturity as affected by 35
TEPLY, LESTER J. See Krehl, Sarma, Teply and Elvehjem 85
Thiamine, added, mental response to 283
—— amount of, relation of, in ration of hog to thiamine and riboflavin content of tissue 107
—— and riboflavin content of tissue, relation of amount of thiamine in ration of hog to 107
—— riboflavin, nicotinic acid and pantothenic acid content of colostrum and milk of the cow and ewe 51
—— —— —— —— —— —— pantothenic acid and ascorbic acid content of restaurant foods 755
—— urinary excretion of, four methods for studying, comparison of 635
THOMAS, A. W. See Houk, Thomas and Sherman 609
Tissue, thiamine and riboflavin content of, relation of amount of thiamine in ration of hog to 107
Tobacco-seed oil, nutritive value of 273
—— oilmeal, protein in, nutritive value of 261
TODHUNTER, ELIZABETH N., AND THELMA J. MCMILLAN. The ascorbic acid content of whole blood and plasma of normal rats with evidence of a sex difference 573
Tooth structure, length of life, growth and eye condition, relation of vitamin A intake to 67
Trypan blue and choline, influence of, upon utilization of carotene and vitamin A for liver storage of vitamin A 333
Tryptophane and niacin requirement, dietary, factors affecting, of growing rat 85

UNSATURATED fat acids, essential, and alpha-tocopherol, interrelation of. Covitamin studies. V. 699
Urinary excretion of thiamine, four methods for studying, comparison of 635
Use of mackerel protein in the bioassay test for vitamin A. Studies on the nutritive value of fish proteins. II. 187
—— —— mice for the measurement of the growth promoting quality of proteins 23
Utilization, food, economy of, relation of fat to. I. By the growing albino rat 203
—— II. By the mature albino rat 213

VARIABILITY in the calcium metabolism and calcium requirements of adult human subjects 407
Vegetable crops in relation to soil fertility. IV. Nutritional values of New Zealand spinach 59

—— fats various, diets containing, skimmed milk powder in, nutritive value of, failure of ether extraction to lower. Studies on the comparative nutritive value of fats. VIII. 747
Vitamin A, added, effects of, on the conjunctiva and the level of vitamin A in the blood 471
—— —— and carotene, utilization of, influence of choline and of trypan blue upon, for liver storage of vitamin A 333
—— —— ascorbic acid and spinal fluid pressure relationships in the young bovine 229
—— —— bioassay test for, use of mackerel protein in. Studies on nutritive value of fish proteins. II. 187
—— —— in the blood, level of, and the effects of added vitamin A on the conjunctiva 471
—— —— intake, relation of, to length of life, growth, tooth structure and eye condition 67
—— —— liver storage of, utilization of carotene and vitamin A for, influence of choline and of trypan blue upon 333
—— C content of market milk, evaporated milk, and powdered whole milk 161
—— D_3 and cod liver oil, relative efficiency of, influence of sources of phosphorus on, in promoting calcification in poults 599
—— —— phosphorus and calcium, influence of, upon fat content of skeleton in growing pigs 565
Volume and composition of rat milk, effect of changes in diet on 249

WADDELL, J. See Patton, Marvel, Petering and Waddell 485
WAISMAN, H. A. See McCall, Waisman, Elvehjem and Jones 685
WAKIM, KHALIL G. See Day, Wakim, Zimmerman and McClung 355
Wheat bran phosphorus, availability of, for rat 193
—— germ, corn germ, and some other protein foods of plant and animal origin, proteins of, comparative growth-promoting value of 675
Whole milk, powdered, market milk, and evaporated milk, vitamin C content of 161
WIDNESS, KATHERINE D. See Jones and Widness 675
WILLIAMSON, ANNE, D. M. HEGSTED, J. M. MCKIBBIN AND F. J. STARE. The effect of variations in the level of dietary calcium upon the growth of young rats receiving atabrine 647
WINZLER, RICHARD J. See Deuel, Hendrick, Movitt, Crockett, Smyth and Winzler 747
—— —— —— —— Hrubetz, Johnston, Winzler, Geiger and Schnakenberg 175
WITTWER, S. H., AND H. B. GOFF. Vegetable crops in relation to soil fertility. IV. Nutritional values of New Zealand spinach 59
WYNN, WINFREY. See Haldi and Wynn 525

YDSE, LAURA C. See Bosshardt, Ydse, Ayres and Barnes 23

ZEPPLIN, THERESSA M. See Schweigert, Shaw, Zepplin and Elvehjem 439
Zimmerman, William H. See Day, Wakim, Zimmerman and McClung 355